The Burden and Spectrum of Paediatric Severe Malaria and Aetiology of Dark Urine Syndrome in Eastern Uganda

Thesis

The Burden and Spectrum of Paediatric Severe Malaria and Aetiology of Dark Urine Syndrome in Eastern Uganda

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Abstract

From May 2011 - April 2012, I prospectively conducted paediatric ward admissions surveillance (WSS) and described both the in-patient burden and clinical spectrum of severe *P. falciparum* malaria (SM) in children admitted to Mbale Regional Referral Hospital (Mbale RRH). Furthermore, epidemiology, clinical features and the outcomes of dark urine syndrome (DUS) were described using both retrospective dark urine epidemiological study (REDUES); (FEAST trial ISRCTN 69856593) and the prospective dark urine epidemiological study (PRODUES) nested in the WSS.

Overall 10,208/23,217 (44.0%) children were enrolled in the WSS, a majority 87% were under 5 years of age. Malaria 6,714/10,208 (65.8%) and DUS 1,087/10,208 (10.6%) were among the common diagnoses. Neonatal conditions accounted for 947/9,551 (10.0%) admissions. The in-hospital mortality was high 655 (6.4%); with higher case fatality rate (CFR) 13.2% in neonates compared to 3.7% in older children, P=0.0001.

The WHO criteria for surveillance of severe malaria were fulfilled in 662 admissions or 10% of all malaria cases. The common clinical presentations included respiratory distress 554/662 (83.7%), shock 411/662 (62.1%), clinical jaundice 177/662 (26.7%), severe anaemia 169/662 (25.5%), hyperlactataemia 134/662 (20.3%) and DUS 93/662 (14.0%); features similar to, but also varying from those reported from other settings. The mortality in SM was high 63/662 (9.5%) but consistent with other reports, with higher CFR in patients with multiple features.

In the REDUES, 394/3,170 (12.4%) had DUS with a majority 318/394 (81.0%) presenting to Eastern Uganda. Complications of DUS included clinical jaundice in 256/318 (80.5%) and severe anaemia (Hb <5g/dL) in 238/310 (77.0%). Current malaria infection 147/300 (49.0%) was lower than evidence of recent infection 192/246 (78.0%). G6PD was marginally higher in DUS 35/224 (15.6%) v 53/489 (10.8%) in those without DUS. Mortality at 48hours (10.4% v 8.9%, *P*=0.402) and at 28 days (12.3% v 9.9%, *P*=0.211) was similar in children with and without DUS respectively. PRODUES enrolled 268 strictly defined cases in whom use of antimalarials 129/268 (48.1%) and herbs 52/268 (19.4%) were common. Haemoglobinuria 92/165 (55.8%) and myoglobinuria 59/165 (36.0%), markers of massive haemolysis and muscle cell injury respectively were found; possibly related to the pathophysiology of malaria.

In conclusion, the spectrum of SM in children at Mbale RRH was similar to, but also varied from current descriptions from other sites. The DUS was linked to malaria with innate characteristics and drugs being risk factors.

List of abbreviations

Abbreviation	Meaning
AARR	Annual Average Reduction Rates
AHG	Anti-human Globin
AIDS	Acquired Immunodeficiency Syndrome
APGAR	Activity, Pulse, Grimace, Appearance & Respiration
ARF	Acute Renal Failure
ART	Antiretroviral Therapy
BCS	Blantyre Coma Scale
CBC	Complete Blood Count
CFR	Case Fatality Rate
CI	Confidence Intervals
CM	Cerebral Malaria
CNS	Central Nervous System
CRF	Case Record Form
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
DRC	Democratic Republic of the Congo
DUS	Dark Urine Syndrome
EDTA	Ethylenediaminetetraacetic Acid
EIR	Entomological Inoculation Rates
ELISA	Enzyme-linked Immunosorbant Assay

ETAT	Emergency Triage and Treatment
FBC	Full Blood Counts
G6PD	Glucose-6-Phosphate Dehydrogenase Deficiency
Hb	Haemoglobin
HCC	Hammersmith Colour Chart
Hct	Haematocrit
HiB	Haemophilus Influenza-B
HIV	Human Immunodeficiency Virus
HIE	Hypoxic Ischaemic Encephalopathy
HMIS	Health Management Information System
HRP-2	Histidine Rich Protein - 2
ICD	International Classification of Diseases
IMCI	Integrated Management of Childhood Illnesses
IRR	Incident Rate Ratio
IV	Intravenous
JCRC	Joint Clinical Research Centre
Kg	Kilogram
Km	Kilometre
KWTRP	KEMRI Wellcome Trust Research Programme
LP	Lumbar Puncture
MDG	Millennium Development Goals
mg	Milligram

mg/Kg	Milligrams per kilogram
mm	Millimetre
mmHg	Millimetres of Mercury
mmol	Millimoles
MOH	Ministry of Health, Uganda
MOP	Manual of Operations
MTA	Material Transfer Agreement
MUAC	Mid Upper Arm Circumference
nPRBC	Non-parasitised red blood cells
^{o}C	Degree Centigrade
OR	Odds ratio
P.	*Plasmodium*
PACU	Paediatric Acute Care Unit
PAR	Paediatric Admission Record
PCP	Pneumocystis Carinii Pneumonia
PCV	Pneumococcal Conjugate Vaccine
PEM	Protein Energy Malnutrition
PI	Principal Investigator
PIDC	Paediatric Infectious Diseases Clinic
pLDH	Plasmodium Lactate Dehydrogenase
pRBC	Parasitised Red Blood Cells
PRODUES	Prospective Dark Urine Epidemiological Studies

RBC	Red Blood Cell
RDT	Rapid Diagnostic Test for malaria
REDUES	Retrospective Dark Urine Epidemiological Studies
RPM	Revolutions per Minute
RR	Relative Risk
RSV	Respiratory Syncytial Virus
SA	Severe Anaemia
SAM	Service availability Mapping
SaO_2	Saturation of Oxygen
SEA	South East Asia
SOP	Standard Operating Procedure
SSA	Sub Saharan Africa
TB	Tuberculosis
TIH	Treatment Induced Hypoglycaemia
TMB	Tetramethyl Benzidine
TNF-α	Tumor Necrosis Factor-alpha
UBOS	Uganda Bureau of Statistics
U5	Under 5
URTI	Upper Respiratory Tract Infection
v	Versus
WBC	White Blood Cells
WCC	White Blood Cell Count
WHO	World Health Organization

Dedication

To my wife Harriet and our two lovely children Nissi and Libni.

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Declaration

I declare that the work presented in this thesis is entirely my own except where otherwise duly acknowledged. In addition, this work has not been presented elsewhere entirely or partially for any other degree or professional qualification. I acknowledge the following contributions:

1. Data from the FEAST Trial, where I was one of the six Trial Site Investigators. I applied for access to the relevant data and was given permission from the Trial Management Group (TMG). These data were used as entry point to understanding the question on DUS (Chapter 5).

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4. The blood counts were conducted, under contract, at the Joint Clinical Research Centre (JCRC) laboratory in Mbale using the automated Act Diff 5 at their research unit in Mbale.
5. The team in Mbale Regional Hospital Clinical Research Laboratory conducted parasitology tests including malaria slides and urinalysis for schistosomiasis.

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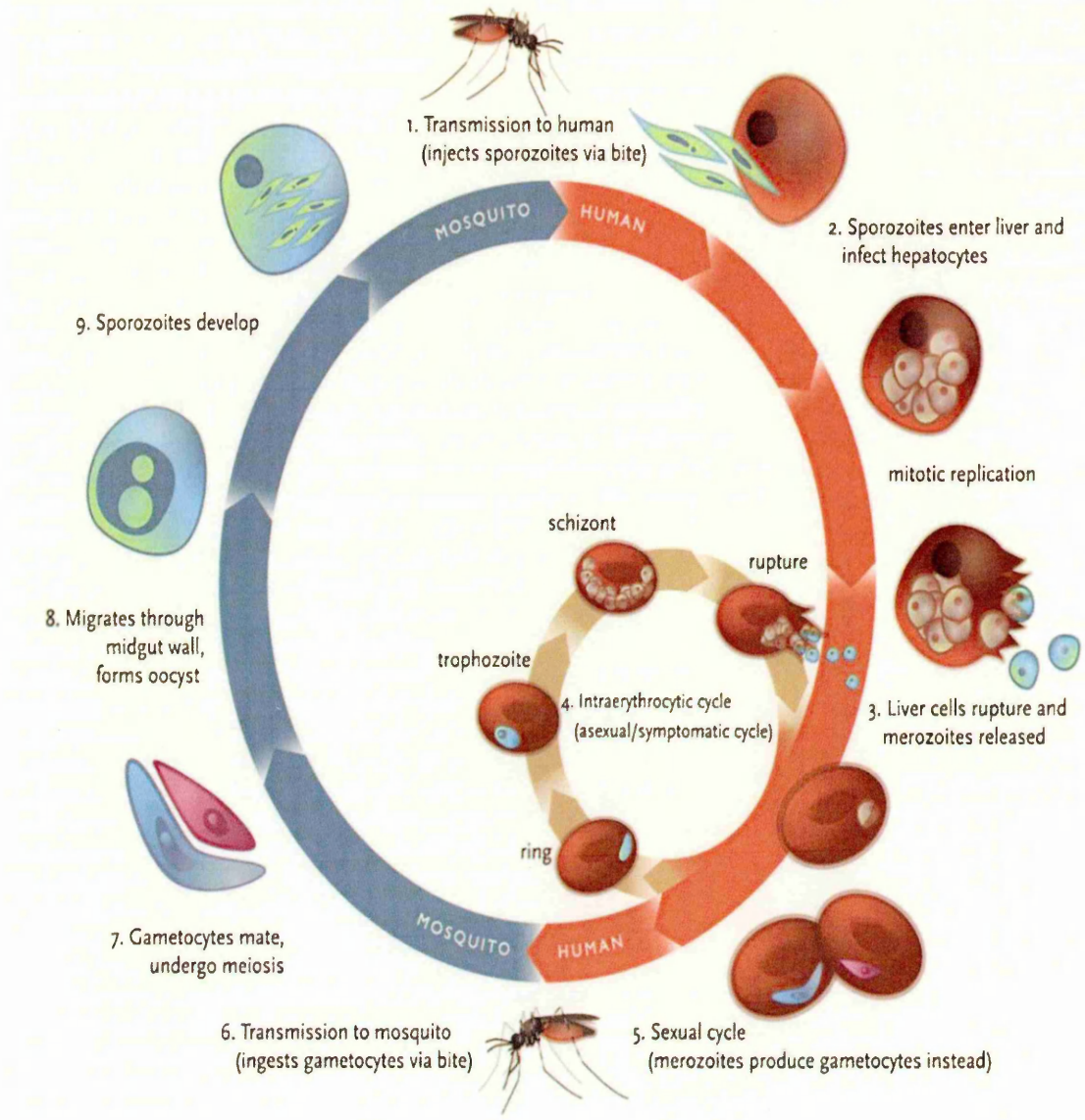
CHAPTER 1: Introduction

1.0. Background

Malaria is a parasitic disease with considerable morbidity and mortality especially in the tropics where it has remained a public health problem [1, 2]. It is caused by protozoan parasites of the genus *Plasmodium* and is transmitted by female *Anopheles* mosquitoes. There are many *Plasmodium* species, but of the five known to infect man - *P. falciparum, P. vivax, P. ovale, P. malariae,* and the recently discovered *P. knowlesi* - the most frequent cause of severe forms of disease associated with poor outcomes is *P. falciparum* [1]. The onset of symptoms follows a 7 to 14 day incubation period when the parasites undergo various developmental changes in the blood and liver [1, 3]. Figure 1.1 below is a summary of the malaria life cycle [4].

Figure 1.1. Life cycle of malaria parasite



Source: *Klein EY. Antimalarial drug resistance: a review of biology and strategies to delay emergence and spread. Int J Antimicrob Agents (2013), http://dx.doi.org/10.1016/j.ijantimicag.2012.12.007 [4]*

Initially, a manifestation of nonspecific symptoms often similar to those of a minor systemic viral illnesses occurs [1]. This early stage of the disease without vital organ involvement is referred to as simple or uncomplicated malaria and readily responds to first line outpatient treatment. However, if ineffective medications are given or if appropriate treatment is delayed, particularly in *P. falciparum* malaria, the parasitaemia continues to build up and pathological involvement of vital organs may result in severe malaria [1]. This development may happen within a few hours. By this stage, the case fatality in people receiving treatment is 10–20%, but rising to almost 100% in untreated cases [1]. This is typically the disease progression in non-immune people especially those under five years old, prime gravidae and travellers from non-endemic areas [1, 5]. In endemic areas, acquired partial immunity may modify progression and outcomes [1, 5]. While an epidemiological map of *P. falciparum* disease [6, 7], and detailed descriptions of its clinical manifestations are still incomplete, commendable progress in the disease expositions has been made. For instance, clearer disease descriptions have been made during the period 1990 - 2014 [8-11], than before 1990 [12]. My work on malaria in this thesis will focus on the clinical picture due to paediatric *P. falciparum* malaria.

Globally, morbidity due to *P. falciparum* malaria accounts for 300 - 500 million clinical episodes with 780,000 direct and 3 million indirect deaths annually [13]. Over 90% of the disease burden is borne by the sub-Saharan Africa (SSA), especially among children [14]. The clinical manifestations and outcomes of malaria have been

described in various settings with marked intercontinental differences [15-18]. For instance, Al-Taiar *et al,* in a prospective observational study describing the clinical presentation and outcomes of severe malaria in children in two public hospitals in Yemen, found that among 808 children who fulfilled the WHO criteria for severe malaria, the three commonest features were respiratory distress 322/808 (40%), severe anaemia 291/800 (37%) and coma 60/808 (8%). The total mortality in this study was 26 (3.2%) of whom the majority 22 (84.6%) had neurological presentations. Prognosticators for a poor outcome included coma, convulsions, female sex and hyperlactataemia [17]. The authors noted that none of the children with uncomplicated severe anaemia died [17]. Findings in this study were similar to those from a number of African series, which noted low mortality in children with uncomplicated severe anaemia [8]; high mortality among patients with impaired consciousness [8] and hyperlactataemia [19], and a rarity of acute renal failure [8, 20]. Nonetheless, there were also marked differences: a high mortality among females was unique to the Yemen study [17, 20]. Within SSA, the clinical manifestations of disease and predictors of poor outcome have shown variation [8, 11, 21, 22]. In this sub-continent there is high in-hospital morbidity and mortality in the under 5 year olds [23]. In-patient mortality among children admitted with severe and complicated malaria varies across different epidemiological settings. For instance, in a cohort study investigating the manifestations and quality of care of children with severe malaria in Mulago Hospital, Kampala, in Central Uganda, Idro *et al* reported a low mortality of 3.1% [24]. In West Africa, Imbert *et al* conducted a retrospective study of

severe malaria comparing the 1990 and 2000 World Health Organization (WHO) criteria for clinical presentation, prognosis and intensive care in Dakar, Senegal, where they reported a higher mortality rate using 1990 compared to 2000 WHO criteria at 17% and 12% respectively [25]. Similar mortality was reported in the coastal area of Kenya at Kilifi District Hospital, where in a prospective study examining the role of hypovolaemia in severe childhood malaria, Maitland *et al* reported an overall mortality of 12.8% and even a higher mortality rate (16%) was noted among infants [19]. In a comparable prospective study examining clinical and laboratory features of childhood severe malaria by Waller *et al* in The Gambia, mortality rates of 15% were reported [26]. Most of the studies reporting malaria related deaths as outcome note that fatalities due to the disease occur mainly within 24 hours of admission [24, 27-29], indicating poor emergency response as well as delays in specific and supportive therapies. Risk factors for fatal outcome include severity and the number of life-threatening complications [27, 28], augmented by inadequate pre-referral primary care and suboptimal care at the referral hospitals [24].

1.1. Definitions of clinical features of severe malaria

Defining severe malaria remains a problem, particularly in children [30]. Before 1990, the diagnosis of childhood severe malaria was predominantly based on two non-overlapping but highly specific syndromes: cerebral malaria and severe malarial anaemia [11, 31, 32]. Over the last two decades, however, it has been increasingly recognized that severe childhood malaria is a more complex syndrome that can

involve multiple body organs and may manifest with a broad range of clinical features [8, 33-35]. This led to the revision by the WHO of criteria for the classification of severe malaria [25]. Against this background, there have been some modifications to the criteria that weigh the frequency of complications and prognosis and its relevance to adult or paediatric models as shown in Table 1.1 below.

Table 1.1. Weighted clinical criteria in children and adults with severe malaria

Prognostic Value[b]		Clinical manifestations or Laboratory findings	Frequency[b]	
Children	Adults		Children	Adults
+	(?)c	Prostration	+++	+++
+++	++	Impaired consciousness	+++	++
+++	+++	Respiratory distress (acidotic breathing)	+++	+
+	++	Multiple convulsions	+++	+
+++	+++	Circulatory collapse	+	+
+++	+++	Pulmonary oedema (radiological)	+/-	+
+++	++	Abnormal bleeding	+/-	+
++	+	Jaundice	+	+++
+	+	Haemoglobinuria	+/-	+
+	+	Severe anaemia	+++	+

[b]On a scale from + to +++; +/- indicates infrequent occurrence, [c]Data not available

Source: *World Health Organization 2000 [36]*

The spectrum of adult type of malaria is different in major ways from that in children. Combining the surveillance for the two different population strata in one criterion

(Table 1.1), therefore, present some limitations. For example, for African children, based upon published prospective studies, a number of clinical manifestations such as renal failure, adult-type respiratory distress syndrome and coagulopathy, are not common [8, 11, 23]. These prospective studies have demonstrated that the majority of the cases of childhood severe disease and the most fatalities comprise of one or more of the three major syndromes including cerebral malaria, severe anaemia or respiratory distress [8].

Until now clinical features strongly associated with mortality of up to 5% are the main criteria used for recognition of severity of malaria. Since the majority of malaria deaths occur within 24 hours of admission [8, 37], this criterion is limited to deaths occurring only during the same admission and not those due to the same illness occurring after discharge. The clinical features for after discharge deaths have not been well studied or documented. The trouble, though, would be the mechanisms to establish the extent to which these deaths were due to the severe malaria and not other causes.

In addition, clinical features responsible for mortality are also responsible for disability, for example coma [38, 39]. Furthermore, length of stay in hospital due to malaria and not logistical issues should be evaluated as criteria for severity especially from the perspective that it takes long to restore derailed physiological processes.

The use of the + or +/- for rating both frequency and prognosis of clinical features is useful for clinical decisions about use of parenteral therapy, and whether or not to

admit a patient to hospital. However, for research purposes, the criteria based on + or +/- are inadequate. Standardization across centres or studies allows results to be compared when taking into account the spectrum of disease and outcome at a given centre or in response to the established or investigational therapy. Quantitative criteria based on mortality rates would be appropriate for use in research. Nonetheless, the use of the WHO criteria for severe malaria has been assessed. Imbert *et al* evaluated the WHO 2000 clinical criteria and found them general and imprecise, although they did find that the WHO 2000 criteria retained high precision for impaired consciousness and symptomatic severe anaemia, similar to that in 1990 criteria. Conversely, the WHO 2000 criteria were simpler to use than 1990 guidelines [25]. Notwithstanding, generalizability of these findings given that these were retrospective data is not feasible, besides, the study was done in an area of low seasonal transmission and that the criteria used were not specific to children [30]. In published prospective studies of African children with malaria [8, 10], manifestations such as renal failure, adult-type respiratory distress syndrome and coagulopathy are uncommon, although, these are frequently described in adults in South East Asian studies [40].

In South-East Asia (SEA), the determinants and distribution of malaria are different from those in the SSA [41]. For instance, there are several ecological types of malaria, including but not limited to urban malaria as reported in India; forest malaria in Thailand and Myanmar; coastal malaria in Indonesia and Southern Thailand [42].

Other human activities have also influenced malaria for example, malaria in rice fields, border and cross-border malaria as reported in Thai-Burma border, malaria in conflict areas [42, 43]. The important determining factors in SEA seem to be mosquito vector and parasite characteristics.

Unlike in SSA, in SEA *Plasmodium vivax* malaria is prevalent accounting for a case burden of over 50% with substantial negative economic impact [42, 43]. However, mortality due to this species is much lower than in *P. falciparum* observed in SSA. Some data from SEA indicates that *P. falciparum* is on the rise and may contribute up to 50% case burden in the region [43]. Though the knowledge in species characteristics for *P. vivax*, lags behind that for *P. falciparum* species, it is particularly interesting to note that different *P. vivax* strains have different incubation periods with some exhibiting long while others short incubation periods [44]. The geographical dominance of the *vivax* species in SEA seem to depend on the incubation period with the long incubation period *vivax* being more prevalent on the international border between the North and South Korea and in some eastern provinces of China [45, 46]. The short incubation period *vivax* that exhibits frequent relapses, that is, Chesson strain; is common in SEA [44].

The SEA region seems to be the epicentre for most antimalarial drug resistance right from chloroquine and sulfadoxine/pyrimethamine to artemesinin based combinations recently, with Thailand and Myanmar as the foci of multidrug resistant *falciparum* [41, 47]. In addition, insecticide resistance has also been reported in the

same region. Both therapeutic drug and insecticide resistance make malaria control an enormous task in the SEA region [48].

In SEA, there is a high prevalence of malaria in specific population groups including ethnic/tribal groups, migrants, rural and urban poor; with risks associated with human behaviours and occupation [48].

Conversely, in *P. falciparum*, a majority of the cases of severe disease and most fatalities have one or more of the complications of cerebral malaria, severe anaemia and respiratory distress (a clinical manifestation of metabolic acidosis) [49, 50]. To date the WHO 2000 criteria with subsequent revised updates in 2006 [51] and most recently 2010 [1], are in use. In the 2010 WHO guidelines [1], severe malaria is defined in a patient with *P. falciparum* asexual parasitaemia and any of one or more of the following clinical and/or laboratory features: prostration (weakness with inability to sit/walk unsupported), impaired consciousness or unarousable coma, failure to feed, respiratory distress (acidotic breathing), multiple seizures (>2 episodes in 24hours), circulatory collapse or shock (SBP <70mmHg in adults or <50mmHg in children), pulmonary oedema (radiological), abnormal spontaneous bleeding, clinical jaundice (plus evidence of other vital organ dysfunction), haemoglobinuria, severe anaemia (normocytic with Hb <5g/dL), hypoglycaemia (<2.2mmol/L or <40mg/dL), metabolic acidosis (<15mmol/L), renal impairment (creatinine >265mmol/L), hyperlactataemia (>5mmol/L), and hyperparasitaemia (>2% or >100,000/μL in low transmission areas, or >5% or >250,000/μL in stable transmission areas). These

criteria exclude patients in whom there is an alternative cause for these clinical features. Moreover, many reports documenting the clinical manifestations of severe malaria have been conducted at research centres based in areas with lower transmission intensity than Uganda [52]. Therefore, the clinical descriptions of severe malaria may still be incomplete. Finally, It has become apparent that severe malaria is often complicated by other co-morbidities [53, 54], and more importantly that these criteria fail to differentiate severe malaria from the sepsis syndromes in children [30], thus lowering their specificity.

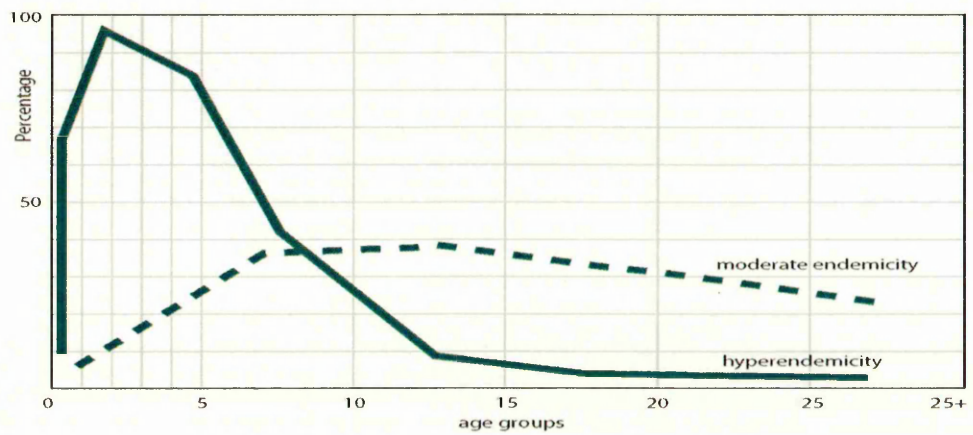
Typically, asymptomatic parasitaemia is common in malaria endemic areas. Clinical diagnostic tools for differentiating critical illness due to malaria, sepsis or other causes [30], are very poor. Owing to limited diagnostic testing and delays in culture positivity (rarely available before 48hours), sepsis is often difficult to diagnose in resource-limited settings, therefore, surveillance at admission may lead to imprecise diagnosis of severe malaria [22, 55-57]. Consequently, severe malaria is likely to be over-diagnosed in patients in malaria endemic areas presenting with life threatening febrile illness [58], and sepsis under diagnosed. With regard to co-morbidity, the role of bacterial infections and chronic illness including HIV and malnutrition [54, 59, 60] or other alternative diagnoses [58], are largely overlooked. But recent evidence suggests that these factors are risk factors to morbidity and outcome in malaria [61]. Despite all the progressive updates and revisions on the defining and supporting criteria for severe malaria in the last three decades, descriptions of the clinical spectrum of childhood severe malaria may still remain incomplete, especially in areas

experiencing hyper to holoendemic transmission. Further prospective descriptions of the manifestations of severe and complicated malaria which consider transmission intensity, seasonality, the effect of varying locations and altitudes, and temporal relationships in the same or different locales are needed to further improve case identification, inform the current criteria and improve on the malaria map.

1.2. The clinical spectrum of severe malaria

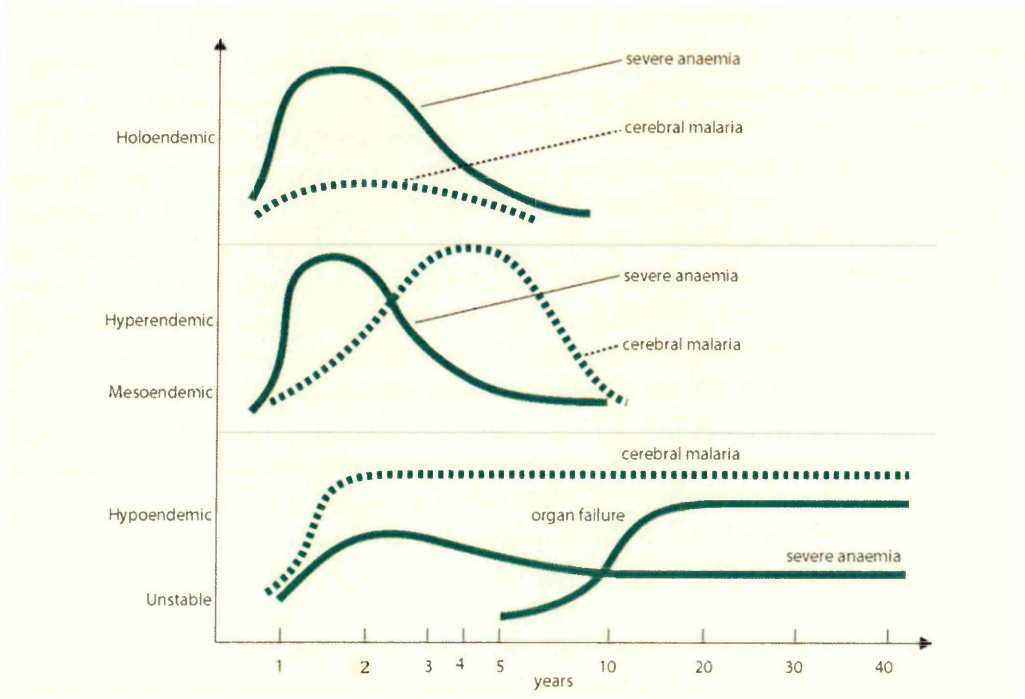
The clinical features of severe malaria in African children have been described over the last few decades and there are clear differences in the disease spectrum, in the tempo and outcome compared to the manifestations in adults as shown in figures 1.2 and 1.3 below. In figure 1.2, in hyperendemic areas, mostly younger children (<5 years) have malaria compared to older children and adults get malaria while in areas with moderate endemicity, older children (>5 years) and adults are affected by the disease. The syndromes of severe malaria affect different age groups in different proportions depending on the levels of endemicity, with organ failure affecting mainly older persons (>5 years) in areas with unstable transmission (figure 1.3).

Figure 1.2. Graphical representation of malaria incident rates between two areas with different transmission



Source: WHO; Global Malaria Programme, Malaria case management. Operations manual 2009 [62]

Figure 1.3. Clinical manifestations of severe malaria with different transmissions



Source: WHO. Global Malaria Programme. Malaria case management. Operations manual 2009[62]

I will consider each clinical feature of severe malaria, and although these are not mutually exclusive, for the purposes of description I will describe one manifestation or syndrome at a time. Almost all severe malaria is related to *P. falciparum*. Some reports indicate that severe malaria also occurs in *P. vivax* but this remains contentious. The scope of this thesis is limited to *P. falciparum* in African children.

1.2.1. Cerebral malaria

In malaria endemic areas, cerebral malaria (CM) affects about 575,000 children <5 years of age annually and is associated with a case fatality rate of 10% - 30% [24, 61, 63] and neurological sequelae [64], including behaviourial abnormalities are reported in 1 - 10% of survivors [39, 65, 66]. In low transmission areas or in areas experiencing an epidemiological transition, CM generally affects older children and adults in whom it is a major cause of mortality [67, 68]. Impairment of consciousness is not always synonymous with 'cerebral malaria'. The consensus international clinical definition of CM includes the presence of unrousable coma in a patient with *P. falciparum* malaria in the absence of other causes of these symptoms [1]. Attendant clinical features including seizures, posturing, metabolic complications (acidosis and hypoglycaemia), clinico-pathological characteristics including specific malaria retinopathy, brain swelling with increased intracranial pressure and alterations in the brain stem functions have been documented [63, 65, 69]. In clinical settings, a broader highly sensitive but non-specific definition of cerebral malaria includes an altered level of consciousness in a patient with malaria [65, 70]. This is mainly because at these settings there is need for a high index of suspicion with the aim of early identification and treatment of the disease so as to improve outcomes. However,

decreased conscious level may result from a variety of metabolic and haemodynamic complications, therefore, this term should be restricted to children with sustained impairment of conscious level (inability to localize pain) after correction of hypoglycaemia, hypovolaemia [8, 19], and sedative effects of anticonvulsants [71]; where applicable. When children with malaria develop coma, it is difficult to differentiate malarial from non-malarial coma. In confirmation of this, postmortem studies in Malawi on the pathogenesis of fatal cerebral malaria indicate that among all fatalities of children clinically diagnosed with cerebral malaria, 23% had died of causes other than cerebral malaria. The true cerebral malaria fatalities in this study showed parasites sequestered in cerebral capillaries, and 75% of them had additional intra- and perivascular pathology [72]. Moreover, retinopathy was a very good clinical predictor of cerebral malaria [72]. Characteristically, cerebral malaria presents with a 1 - 4 day history of fever associated with convulsions lasting over 10 minutes. Coma is frequently precipitated by a prolonged seizure [31] or hypoglycaemia [73]. A number of underlying disease processes and mechanisms have been proposed. The most reported pathophysiological process supported by postmortem studies [72], is the sequestration of mature *P. falciparum*-parasitized red blood cells (schizonts) in the microcirculatory vasculature of the brain and other vital organs [72]. Sequestration involves cytoadherence of parasitized red blood cells to the vascular endothelium of post-capillary venules (low flow areas) [74]. Other pathological mechanisms that contribute to localization include the role of inflammatory cytokines [75], and the total parasite biomass [76]. Rosetting has been demonstrated in a number of studies

to contribute to cerebral malaria. In this phenomenon parasitized and non-parasitized RBCs stick together, a mechanism thought to contribute to low flow in the microvasculature [77-79]. On the other hand, agglutination, in which parasitized RBCs stick together to form goblets, has also been suggested as an underlying mechanism [80]. In addition to these, mechanical impediments to vascular flow by deformation of non-parasitized RBCs have also been shown to be associated with severe disease, and may contribute to a mechanical 'hypothesis' of the aetiology of severe malaria by affecting their ease of maneuverability through small diameter blood vessels [81]. Furthermore, deformed RBCs are highly vulnerable to lysis and splenic clearance contributing to the anaemia associated with severe malaria [81]. Lastly, the theory on the role of cytokines has been put forward [82]. Some studies have demonstrated increased production of various cytokines in response to severe malaria [83]. In cerebral malaria, several studies have demonstrated increased production of tumor necrosis factor alpha (TNF-alpha)[84, 85], which in turn enhances the process of endothelial cytoadherence, release of nitric oxide (NO), hypoglycaemia and bone marrow dysfunction [86]. Studies have shown that NO is toxic and interferes with the nervous system and hence is contributory to the pathogenesis of coma [87, 88]. Within the group of children presenting primarily with coma there is considerable heterogeneity [65, 72]. In the majority of cases, rapid provision of supportive therapies and the administration of an appropriate antimalarial (IV Artemesinin based drugs or Quinine) is accompanied by resolution of coma over the subsequent 4 - 48 hours, but earlier recovery has been registered in

children than adults [89-91]. In the AQUAMAT study [61], among the subgroup with coma outcome was not different in quinine and Artesunate arms and the overall mortality was 19%. Nonetheless, there are a number of children who, at presentation, are indistinguishable from children of good prognosis but then develop persistent deep coma, status epilepticus, posturing and brain stem features suggestive of a raised intracranial pressure. Such children pose a significant therapeutic challenge since they often require multiple anti-convulsant medications, with the attendant risk of depression of respiratory drive which, in turn, will worsen brain swelling. The most common seizures in severe malaria are focal motor or generalised tonic-clonic convulsions [67, 68, 92]. However, about 25% of seizures are subtle or sub-clinical (detected only with EEG), frequently manifesting as eye deviation, an irregular respiratory pattern or drooling [92]. Intracranial pressure (ICP) monitoring and post-mortem studies in this group of patients with prolonged and complicated course have demonstrated that brain swelling is a major feature in fatal cases [92, 93], particularly in the agonal stages. In one study, the only sign found to be associated with the development of intermediate or severe intracranial hypertension was a sluggish or absent pupillary response [92], other signs such as absent extensor motor response, pupillary dilatation, decerebrate posturing, or absent oculocephalic reflexes were not [92]. Mannitol is effective in lowering the ICP, but its short effect precludes its choice as empiric regimen. A recent double blind randomised controlled trial (RCT) of mannitol v placebo in Mulago Hospital in Uganda indicated that time to regain consciousness (P=0.11), sit unsupported (P=0.81), time to start oral intake

(P=0.13) and total coma duration (P=0.07) were not different in the two study arms and mortality in the placebo (13/80 or 16.3%) and mannitol (10/76 or 13.2%) groups were similar RR=1.2 (CI 0.5-2.7) [94]. Even when used in association with intracranial pressure monitoring, mannitol did not prevent the development of intractable intracranial hypertension in those children with a poor outcome [92]. Factors associated with the development of neurological sequelae include protracted seizures, prolonged deep coma and hypoglycaemia [67, 95].

1.2.2. Posturing

There are three classical abnormal postures noted in childhood cerebral malaria: decorticate, decerebrate and opisthotonus. The aetiology of posturing remains poorly described but it indicates direct brain involvement in severe malaria [96]. Typically any of these manifestations often follows prolonged convulsions, coma and features of increased intracranial pressure [31, 97]. A patient with a decorticate posture has gross extension of the neck, trunk and lower limbs with tensed muscle tone leading to stiffness, flexed elbows, clenched fists and hands bent toward the chest [36]. On the other hand, decerebrate posturing is a central nervous system (CNS) motor manifestation in which a patient has extension of the upper limbs, adduction and internal rotation of the shoulders, with pronation of the forearms, commonly described at the bedside as "waiters' gaze" [36]. Similarly, opisthotonus posturing is defined when a patient has gross extension of the neck, trunk and lower limbs with rigidity [36]. Occasionally patients manifest with more than one posturing type [96],

though, reasons for these combinations of different postures in particular patients have not been well described. However, it is plausible to assume that they are as a result of a mixture of intracranial pathology including micro-haemorrhage, increased intracranial pressure, compression of brain base and brain swelling since posturing has been associated with these features [38, 96]. Idro *et al* found that the frequency of posturing was lower in the younger age group compared to older children owing to the open fontanelle or soft cranial sutures, which easily accommodate increased intracranial pressure [96]. Several risk factors similar to those in cerebral malaria have been associated with posturing associated with increased intracranial pressure and metabolic derangements [63, 96, 98]. The clinical significance of these risk factors in this condition remains poorly described. Also, no genetic or epidemiological studies have described reasons for selective posturing in some children with cerebral involvement and not others.

1.2.3. Recurrent convulsions

Recurrent convulsions are defined when on admission a patient has had 2 or more generalized convulsions within the 24 hours prior to admission [1]. Excluded in this category are seizures accompanying fever in children who have suffered a previous non-febrile seizures [1, 99], because of other possible aetiologies of convulsions in them; such as an preexisting diagnosis of epilepsy. The genesis of convulsions is multifactorial including cerebral malaria, hypoglycaemia, hyperpyrexia and metabolic abnormalities [100, 101]. In Kilifi, Kenya, Idro *et al* conducted a prospective

study on incidence, aetiology and outcome of acute seizures and found 58% of the seizures were associated with malaria in children aged above 6 months and that 57.1% of patients with status epilepticus had malaria [102]. Earlier, the same investigators showed that recurrent convulsions had a role in persistent neurological and cognitive impairment among children [38]. Prevention of seizures in cerebral malaria has been tried. In a randomised controlled trial (RCT) of I/M phenobarbital 20mg/kg v placebo for preventing seizures in childhood cerebral malaria in Kilifi, Kenya, Crawley *et al* showed that seizure frequency was significantly lower 18/170 [11%] in the phenobarbital group than in the placebo group (46/170 [27%]; OR 0.32 [95% CI 0.18-0.58]). Nonetheless, the authors noted that mortality was more than twice in the phenobarbital group 30/170 [18%] v 14/170 [8%] in the placebo group; OR 2.39 [1.28-4.64]. Excess mortality was largely due to respiratory adverse events which were greatly increased in children who received phenobarbital [103]. Similarly, Gwer *et al* in a RCT to investigate the use of fosphenytoin (n=85) v placebo (n=88) for seizure prevention in African children with coma of infectious origin recruited 173 subjects of median age, 2.6 years [IQR 1.7-3.7], distributed into three groups according to aetiology including; 110 with cerebral malaria, 8 with pyogenic meningitis, and 55 with unclassified infectious aetiology. The authors concluded that there were neither differences in seizures nor neurologic deficits prevention between the two study arms (fosphenytoin v placebo) among children with coma of infectious causes [104]. Other than prevention, a number of studies have evaluated use of short acting anticonvulsants for the control of convulsions. Ogutu *et al* reported that

diazepam is readily bioavailable following I/V administration but does not control all convulsions [105]. Similarly, Muchohi *et al* evaluated midazolam for the control of seizures and found its efficacy was poor at the current recommended doses [106]. However, when evaluating both diazepam and midazolam, Doshi found that midazolam was more bioavailable and better in controlling seizures in children with non-malarial causes than diazepam [107]. Whereas, genetic susceptibility to convulsions have been reported in viral-related seizures [108], similar studies in malaria are still lagging behind.

1.2.4. Severe malarial anaemia

The WHO defines severe malarial anaemia (SMA) as normocytic anaemia with haemoglobin (Hb) concentrations <5.0g/dL [or packed cell volume (PCV) <15%] irrespective of the level of parasite density [1]. This definition has high sensitivity but a low specificity, ideal in clinical settings in resources limited settings where practically assessing and closely monitoring for complications due to SMA is largely not feasible. In these settings there is need for reducing mortality due to SMA. In addition, providing for either Hb or PCV reference is pragmatic since obtaining an Hb result is less expensive, but also less hazardous for health personnel compared to PCV. The typical case of SMA is an infant presenting with a 3 to 4 day history of fever, signs of pallor, lethargy and a haemoglobin concentration between 4 - 6g/dL with or without evidence of respiratory distress. In the SSA, SMA is commonly a disease in children <5 years of age [109, 110], in whom an estimated 90% of the

800,000 severe anaemia-related deaths occur annually [111, 112]. Most of these deaths occur within 24 hours of admission, often in the process of waiting for specific and supportive therapeutic interventions [113-116]. The prevalence of SMA in African children coming to hospital is 21.2%, with a wide variation of 7.5 - 34% between countries and regions [117]. In-hospital mortality in complicated anaemia is high (9 - 30%) [116, 118, 119], with additional 12% post discharge mortality in 3 to 6 months and re-hospitalisation rates of 6% [120]. Earlier and recent studies indicate that SMA is not homogenous within the under 5 year olds [116, 118, 119]; in whom prognosis is worse with younger age [121]. In stable high transmission areas, the highest risk for SMA is in infancy while in areas with moderate to low transmission, the risk peaks at 24 months of age and generally decreases after 5 years of age [122]. Their level of consciousness is often impaired and coma develops in a number of them. Idro *et al* in Mulago hospital in Kampala found that severe anaemia was independently associated with profound coma OR 1.34 (CI 1.17 - 1.95; P=0.002) and younger age; <3 years OR 1.42 (CI 1.13 - 1.54; P=0.001) [123]. Earlier clinical descriptions of complicated SMA were confused with and subsequently managed as biventricular heart failure [124]. More recently, however, studies have indicated that in a majority of children, respiratory distress is due to severe lactic acidosis [19, 116, 125], resulting from hypoxia and clinical shock [55, 126]. Since then, less is reported on congestive heart failure complicating SMA. Despite progress in clinical descriptions in SMA, the pathophysiology of SMA remains poorly understood for a number of reasons. Firstly, the temporal relationship between parasitaemia and clinical disease is difficult to

establish. For instance, while some individuals remain asymptomatic with parasitaemia, others develop severe anaemia when the parasitaemia has cleared [116, 127, 128]. Recently, delayed haemolysis leading to anaemia secondary to artemesinin-based antimalarials has been described in some patients exposed to malaria [129-131], but similar studies have not been done in African children. Nonetheless, both asymptomatic and symptomatic malaria parasitaemia are associated with anaemia ($P<0.001$) [113]. Furthermore, factors related to which groups of children develop a specific type and degree of inflammatory responses in severe anaemia remain poorly understood. Nonetheless, some plausible mechanisms have been suggested to play a role singly or in combination [132, 133]. These include haemolysis of parasitized red blood cells (pRBC) and non-parasitized red blood cells (nPRBC) [134, 135]. In addition, haemozoin, a by-product of haemoglobin catabolism by intra-erythrocytic malaria parasites [136], has been linked to the pathophysiology of severe malaria. Since its discovery in 1950s, studies of haemozoin in malaria infections [137], indicate its role in toxic suppression of the bone marrow resulting in anaemia [138]. Whereas, earlier reports indicated that high mortality was associated with high levels of circulating haemozoin, in a large multicentre study in six sites (Banjul, Blantyre, Kilifi, Kumasi, Lambarene and Libraville) with varying transmission intensity across Africa, Kremsner *et al* did not find a positive correlation between high circulating haemozoin and mortality. However, they found a negative correlation between high levels of haemozoin and haematocrit levels [139]. On the other hand, the role of the spleen especially in sequestration of RBCs resulting in severe anaemia is well known

[140]. In a prospective study in Mbale Hospital in Uganda on spleen volume and clinical disease manifestations of severe malaria in African children, we found that the spleen size in SMA was larger than for children with CM [141], suggesting both a mechanical and an immune related function of the spleen in relation to SMA in an area of high malaria transmission.

The WHO recommends blood transfusion for patients with Hb of <5.0g/dL in holoendemic areas and <7.0g/dL in low transmission areas [1]. The concern with these guidelines is that many children would qualify for a blood transfusion but the majority of those with asymptomatic anaemia, known to be associated with low mortality <1%[8], would not benefit from these transfusions. Children in the SSA are amongst the chief recipients of transfused blood [114]. In many malaria endemic areas between 19 - 50% of children admitted to hospital receive a blood transfusion, principally for SMA [113, 122, 132]. Although transfusion may be lifesaving, the lack of adequate blood supplies to meet the high demand, the lack of robust blood screening services, inadequate haemovigillance and quality assurance, the risk of transmission of HIV or other blood borne infections and of adverse reactions [142], still remains a reality. Furthermore, in many hospitals in the SSA, blood is in desperately short supply mainly because of very high demand for blood transfusions among children compared to the developed countries where increased demand of blood transfusions is for the elderly [142]. In the absence of respiratory distress or coma, in-hospital mortality is relatively low (<1%) in children with SMA [8, 113].

Currently there are insufficient data to be sure whether routinely giving blood to clinically stable children with profound anaemia in endemic malarious areas reduces immediate and/or long-term morbidity and mortality.

1.2.5. Hypoglycaemia

Hypoglycaemia, defined as a blood sugar <2.2mmol/L [1], is a common complication of severe *falciparum* malaria across all age groups and epidemiological settings. It is reported in 8% of adults with severe malaria in SEA, in up to 30% of African children and in 50% of pregnant women [73, 102, 143, 144]. Hypoglycaemia accounts for 153,000 - 269,000 malaria related deaths in Africa [73, 102, 143, 144]. Pragmatically, hypoglycaemia may be classified as primary or secondary (treatment-induced). Treatment-induced hypoglycaemia (TIH) frequently results from rapid infusion of quinine in adults, but is unrelated to quinine-dose in African children [73, 145]. The aetiology of hypoglycaemia includes parasite glucose metabolism, poor food intake by patients, abnormalities in the metabolic pathways and hyperinsulinaemia induced by quinine [146-148]. Whereas adult hypoglycaemia may often follow quinine therapy, TIH seems a rare phenomenon in children [149]. Primary hypoglycaemia is the most common form and tends to be associated with other overlapping clinical features of severe malaria including coma, repeated convulsions, shock and hyperparasitaemia [143]. In cerebral malaria, it is responsible

for poor outcomes with 20 - 40% mortality [65, 149]. Recurrent hypoglycaemia is now established as a poor prognosticator in severe malaria with up to 71% mortality [145]. In a recent prospective study of 437 consecutively recruited children with severe malaria in Mali, Willcox *et al* reported higher mean blood glucose level among survivors than those who died (7.6mmol/L v 4.6mmol/L; $P<0.001$). In addition, the odds of death in this study group were significantly higher among those with hypoglycaemia [OR (95%CI) 11.9 (2.1 - 67.0) v 5.2 (1.9 - 14.6)]. Furthermore, these investigators found that within a group of patients with low glucose concentration (2.2 - 4.4mmol/L), high mortality was associated with a higher glucose level threshold than the current WHO definition of hypoglycaemia at ≤2.2mmol/L [1, 150]. Poor outcomes have also been reported in children with hypoglycaemia in multiple other sites across Africa [19, 73, 149, 151]. It is noted that hypoglycaemia is singly associated with a poor outcome in children, and mortality is even higher when it overlaps with coma, acidaemia (pH<7.3), or hyperlactataemia (lactate >5mmol/L) [152-154]. The emergency treatment of hypoglycaemia in children is an intravenous infusion of 25% dextrose dosed at 2mL/Kg. However, patients with both impaired consciousness and hypoglycaemia may respond to this treatment in various ways. For instance, there are those patients who regain consciousness with good outcomes, and others who do not, suggesting multiple aetiologies in the syndrome of impaired consciousness. Ogetti *et al* in their study on hypoglycaemia in severe malaria in association with treatment found that post treatment hypoglycaemia was frequent (66%) among euglycaemic children at admission, whilst 34% had hypoglycaemia at

admission. In addition, they found 52% of the children had a single episode whilst the remaining 48% had multiple episodes. In addition, they reported that the odds of having an episode after admission was high, OR = 3.18 (CI 2.21 - 4.58; $P<0.0001$). Significantly, however, post treatment hypoglycaemia was associated with poor outcomes compared to euglycaemic patients since mortality was 24% v 8%, OR = 3.45 (95%CI 2.30-5.16); $P<0.01$ [73].

1.2.6. Respiratory distress

The WHO guidelines included both pulmonary oedema and deep acidotic breathing in the definition/cause of respiratory distress [1]. Nonetheless, in children, pulmonary oedema is rare [116], and this is more applicable for the adult acute lung injury or secondary to fluid management. Deep acidotic breathing in the same criteria is more applicable to children [8, 127], in whom it is a common manifestation of severe malaria [155]. More importantly, as recently indicated [19], and previously in another study [28], it is a major independent risk factor for fatal outcome. Whilst recognized for many years as complication of malaria, only recently has its aetiology been better understood. Formerly, it was assumed to be due to biventricular heart failure (pulmonary oedema) or direct lung injury as seen in adult studies [1, 128]. Studies in multiple sites across Africa have now shown evidence that respiratory distress is deep breathing (also known as 'Kussmaul's breathing) and is a secondary compensatory mechanism for metabolic acidosis [8, 19, 26, 156, 157]. Respiratory distress is one of the top three commonly recognizable clinical features of the disease

with poor outcome [8, 156]. Studies in African children with severe malaria associate respiratory distress with acidosis and hyperlactataemia [158], and this is not associated with adult type respiratory distress. The case fatality in the paediatric type respiratory distress is high. Marsh *et al* reported the prevalence of respiratory distress among children with severe malaria at 13.7% (n = 1,833) with a CFR of 13.9% (n=251) [8, 127]. It often exists with other manifestations of severe malaria including severe anaemia [113] and cerebral malaria [123], in which higher case fatalities have been reported.

1.2.7. Acidosis

Metabolic acidosis is best defined by an abnormal base deficit ≥8mmol/L [159] or the presence of plasma bicarbonate of <15mmol/L [1, 19]. It is an important laboratory criterion for clinical definition of severe malaria [8, 156, 160]. Moreover, it is an independent prognosticator of poor outcome in both adults and children [8, 156]. Physiological studies in African children have indicated that metabolic acidosis and its clinical correlate (respiratory distress) is associated with clinical and physiological features of volume depletion or shock [19]. Mortality in severe malaria was directly proportional to the severity of shock [19, 159]. Studies in Ghana showed that prolonged capillary refilling time (CRT>2s), a clinical feature of shock, was an important predictor of fatal outcome [161]; making it a single most important prognosticator of deaths in severe malaria [19]. Some authors have indicated that acidosis is a marker of metabolic dysfunction [160, 162, 163], whilst others have

suggested that it is related to microcirculatory impairment secondary to sequestration [164, 165]. Another marker for acidosis is increased serum lactate (>5mmols/L) which has been shown to be associated with poor outcome [146]. Increases in lactate, due to anaerobic respiration, may be transient for example during a convulsion or exercise and has a relatively good prognosis. Conversely, hyperlactataemia and metabolic acidaemia with acid base derangements reflected in changes in pH, base deficit and bicarbonate are associated with poor prognosis [166].

1.3. Blackwater fever (BWF)

The syndrome of "blackwater fever" is uncommon in the modern descriptions of paediatric malaria in Africa [167]. In adults, the syndrome has been described in case studies and series since the 18th century [168-171]. At the turn of the 20th century, the aetiology of the syndrome was further described but these elucidations remain incomplete. Studies since then attributed this syndrome to non-immune persons residing in Africa [170, 172]; who had poor adherence to quinine prophylaxis for malaria and often took incomplete doses of quinine during an attack of the disease in the 1940s [170]. The plausible causal association between quinine in the 1940s and the condition was reinforced by the observation that the syndrome became rare in the 1960s when the use of quinine became increasingly replaced by Chloroquine [170]. Other risk factors include genetic predisposition especially in haemolytic conditions. Glucose-6-phosphate dehydrogenase deficiency (G6PD) has been associated with massive haemolysis among malaria patients, use of quinine or other quinolone drugs

for treatment of malaria [168, 173-176]. In addition, dietary factors such as fava beans have been associated with haemolysis in G6PD [171]. Other causes of increased intravascular haemolysis that have been implicated in haemoglobinuria include incompatible ABO blood group transfusions [177], *Salmonella typhii* septicaemia [178] and envenomation, especially by snakes and spiders [175]. Despite these descriptions and observations, until recently, characteristic BWF has been considered rare among children with severe malaria in SSA [27, 28, 168-171]. In the recent literature, however, some descriptions have been published. In a study involving 251 Nigerian children with severe malaria, Ajetunmobi *et al* reported BWF in 48 (19.1%). To my knowledge, this is the second highest prevalence reported following another study among 378 children in DRC, where Kunuanunua *et al* reported BWF in 89 (23.5%) [179]. Both Ajetunmobi *et al* [180] and Kunuanunua *et al* [179] reported that a majority of children with SM were <5 years (76.8% and 51.3% respectively). Moreover, in both studies, patients with BWF had a higher median age compared to those without. However, Ajetunmobi *et al* reported that there were no differences noted in gender, nutrition status or parasite density. In addition, these authors found that jaundice was significantly associated with haemoglobinuria $P<0.001$. Unlike other reports on childhood BWF in Africa, Ajetunmobi *et al* reported a high rate of renal failure 3/48 (6.3%) compared to none in those without BWF [180]. Some aspects of the classical adult BWF especially evidence of asexual forms of *P. falciparum* malaria, intravascular haemolysis, anaemia and jaundice [167, 170], have been described either separately or as part of the childhood severe malaria spectrum [27, 32]. Even fewer data have

shown renal involvement in childhood BWF or severe malaria [61, 180], compared to similar studies in adults [175, 181, 182]. In general BWF in adults is associated with low mortality [168-171], unless accompanied by other features of severity [167]. There are few similar studies reporting outcome from BWF in African children. There may be different pathophysiological processes in the two age strata. Some studies on children with dark urine are ambivalent on its aetiology or pathophysiology. For example, recurrent *P. falciparum* malaria and frequent use of quinine in childhood have been associated with childhood blackwater fever [183]; but these can be interpreted to mean low malaria clinical immunity in childhood [172], and toxicity of quinine [183] respectively. Furthermore, two biologically different proteins have been isolated in children with dark urine in severe malaria: haemoglobin and myoglobin [184], but with possible different pathophysiologies. Haemoglobinuria, a marker of severe haemolysis is mainly associated with acute intravascular haemolysis [183, 184], while myoglobinuria [185], manifests mainly among children with cerebral malaria and hyperlactataemia; suggesting hypoxic muscle cell injury from sequestration of parasitized red blood cells [183]. This picture of illness and background would indicate no single aetiology and/or pathophysiology of childhood BWF since most literature suggests a multi-aetiological and pathophysiological process. The literature on BWF with significant prevalence of the syndrome in Africa and Asia is summarised in Table 1.2 below. In publications from some hospitals in Europe, the study participants mainly were exposed to malaria in Africa as expatriates, missionaries or residents in Europe from Africa and Asia returning from visits home.

Table 1.2. Summary of studies on Blackwater fever in Asia and Africa

Author, study site and period	Malaria endemicity, Study design & sample size	Aims & findings	Aetiology of BWF & outcomes
Hue *et al* [186], Ho Chi Minh City, Vietnam. 1993 - 2001.	Malaria hyper endemic area. Case Control study with: Cases N=82 Control N=524 healthy people	To determine spectrum of G6PD mutant variants and their relationship with haemoglobinuria. Ninety-two per cent of participants with G6PD and haemoglobinuria were males. Haemoglobinuria was strongly associated with G6PD mutations OR 15; 95% CI (7.7 - 28.9) $P<0.001$.	G6PD, malaria & antimalarials.
Lau *et al* [187], Tuen Mun Hospital, Hong Kong. March 1993 - Feb 2005.	Malaria low endemic area. Retrospective review. Cases N=6	To describe recurrent haemoglobinuria in children. All participants were Males, mean age 5.5 years with G6PD. Hb at admission 3.5 - 8.4g/dL. Each patient had 7 episodes of dark urine	G6PD. All 6 survived. 5 had blood transfusions. 2 developed acute renal failure
Jamaiah *et al* [188], Kuala Lumpur, Malaysia, South East Asia. 1994 - 2003.	Seasonal malaria endemicity. Retrospective study. Cases N=86 adults, 60 (70%) males.	To assess the prevalence and describe clinical features among patients presenting with severe malaria to a tertiary hospital. Age range (20 - 39) years, Severe anaemia and jaundice 77%. Blackwater fever 13%. Cerebral malaria 10%.	Severe malaria study, Male predominance; only 13% had BWF. 83 survived, 3 died
Tran *et al* [189], Ho Chi Minh City, Southern Vietnam. April 1993 - May 1994.	Malaria hyper endemic area. Prospective study Cases N=50 of whom 47 male and 3 female.	To describe epidemiology of BWF in Vietnam. 50 cases of blackwater fever, mean age 30.5 years, G6PD 54%, Quinine use 56%, malaria infection 32%. 30% had previous BWF episode, 8% had family history of BWF. Jaundice 80%, 96% had hyperbilirubinaemia.	Quinine, G6PD, malaria. One fatal outcome.

O'Donnell *et al* [184], Papua New Guinea. October 1993 - February 1996	Malaria hyper endemic area. Prospective study. 351 children (51.9% male) with severe malaria: 22 had dark urine with haem protein, 99 normal colour urine with haem protein and 236 had normal urine.	To describe the red cell variants among children with severe malaria. Mean age 2.8 years, Spectrum of severe malaria was cerebral malaria, hyperlactataemia and severe malarial anaemia. Plasma myoglobin was greater in cerebral malaria, hyperlactaemia.	Malaria, No deaths, no acute renal failure. Increased fragility of RBCs in alpha - thalassaemia.
Delacollette *et al* 1995 [170]. Kivu Mountains Zaire. January 1985 - March 1986.	Malaria hyper endemic area. Prospective study. 38 males studied.	To describe the aetiology of haemoglobinuria in DRC. Of the 38 patients, 50% were none immune. G6PD 10.5%, Leptospirosis 5.3%, Hantaan virus 5.3%, Malaria 73.7% all had low parastaemia. Quinine was strongly associated with BWF among patients with malaria $P<0.001$	Malaria, G6PD, Hantaan viral infection, Leptospirosis. All survived, no renal failure
Bodi *et al* [190], Kinshasa, DRC. January 2010 to December 2011.	Malaria hyper endemic urban area. Case Control study. Cases, N=43 Haemoglobin in the urine of febrile patients with jaundice, anaemia and confirmed *P. falciparum* malaria. Controls, N=86 uncomplicated malaria according to 2011 WHO definition) matched for age, sex and place of residence	To investigate risk factors among children with BWF in Kinshasa, DRC. Seasonal association 38(88.4%) cases occurred in wet season v 51(59.3%) controls Cases 8.62 years SD3.8 Control 8.55 years SD3.8 Sex ratio in both groups was similar. G6PD present in 8(18.6%) cases and 34(39.5%) controls. High LDL levels >400 in 9(20.9%) cases v 10 (11.6%) controls. Hb <10g/dl present in 33(76.7%) v 10 (11.6%) controls.	Entry criteria restricted study to participants with malaria parasitaemia. Logistic regression analysis showed that ingestion of quinine, low parasitaemia and rainy season were the main risk factors 41(95.3%) cases had consumed quinine v 26(30.2%) controls. 7(16.2%) cases developed acute renal failure (ARF),

		High creatinine present in 7(16.3%) case v 0 in controls. No association with blood group type. No apparent association with malaria species (*falciparum* or *malariae*); however low-density parasitaemia more common in cases 32(78.0%) v controls 43(51.8%).	
Daubrey-Portey *et al* [175], Abidjan, Ivory Coast, West Africa 1996 - 2000	Low seasonal malaria transmission. Prospective study. Cases N=41 Males =31 Females =10	To identify antimalarials associated with BWF. A case series of 41 adolescents and adults, 80% had exposure to quinine. Mean age 36.3 years	Quinine and other related molecules. 90% developed acute renal failure, 20% deaths and 47% survivors had renal dialysis.
Gobbi et al [183], Burundi highlands, Burundi. 1992 - 2002.	Malaria hyper endemic area. Prospective study. Cases, N=9 Children with malaria on treatment with quinine	To describe pathogenesis and management of haemoglobinuria in children. All 9 cases were males, mean age 8.2 years severely anaemic (Hb <4.5g/dL or <6g/dL with dyspnoea. All had blood transfusions (4 once and 5 re-transfused in the same illness.	Quinine. All 9 recovered well. No acute renal failure The major limitation of this study was that G6PD was not measured.
Rogier *et al* [172], Dielmo, Senegal. 1990 - 2000.	Malaria hyper endemic rural area. Sub group on a Prospective community study (N=315). BWF reported in 3 cases (2male and 1 female).	To describe case presentation of BWF in African children. 142 children were followed over a period 3 years. BWF in this cohort was rare in Senegal. These 3 children suffered from recurrent malaria every 4-6 weeks for long period. Used quinine for treatment.	Malaria, quinine. 1/3 died. None was in renal failure

Ajetunmobi *et al* [180], Ibadan, Nigeria. 21 months study.	Malaria hyper endemic area. Prospective descriptive study. N=251; children with severe malaria	To describe clinical presentation of childhood severe malaria in Nigeria. 76.8% children <5 years. Older children with BWF. 48/251 (19.1%) with BWF. Median for severe malaria =35 months and for BWF 52.5 months	19% of children with severe malaria had BWF. Possible role of quinine. 3/48 (6.3%) with ARF. All survived.
Kunuanunua *et al* [179], Kinshasa, DRC. 1st January – 31st December 2008.	Malaria hyper endemic rural area. Prospective cohort study. N=378 children with severe malaria.	Describe severe malaria in children presenting with haemoglobinuria. 51.3% <5 years, female: male ratio 1:1.5. ARF in 89 (23.5%) of which 87 (97.8%) had BWF.	In children with severe malaria 87/378 (23.0%) developed BWF. Malaria, ARF 23.5%, mortality in ARF group was 12.6%.
Ekvall *et al* [191], Kisarawe District Hospital, Coastal Tanzania. June – August 1997.	Malaria holoendemic area. Prospective study. 20 children.	To describe acute haemolysis in children with hyperparasitaemia. Heavy *P. falciparum* malaria. Plasma Hb <1% of blood Hb, Urine <0.5% of the total Hb and haemoglobinuria in 14/20 children. All patients had increased complement C3c fragments an autologous IgG. 20 months	Malaria. No organ dysfunction noted

From the above table BWF is a condition of the tropics especially Africa and Asia. The literature is varied but few studies were designed to allow conclusions about aetiology of BWF with some studies reporting exclusively on severe malaria, others exclusively on cohorts of G6PD deficient patients. From those reporting exclusively on BWF, malaria is not always present; but few studies indicate history of recent ingestion of antimalarials. On use of Mefloquine and Artemesinin, a large community based clinical trial in the Thai/Burma border indicated that only 3 children developed BWF, though the parasite density in these children was very high (104,000 - 790,000) [192]. Most of these studies have not reported on mortality, morbidity including blood transfusion, use of antimalarials, renal impairment or acute renal failure. In addition these studies have not reported on length of hospital stay. BWF data from Europe have reported on study participants who had recent travel in Africa or SEA.

The complications and outcome of BWF in African children seem to be different from those in adults since renal failure and mortality are higher in adults [175, 193]. Lastly, the aetiology of BWF in African children seems to differ from that in Asian children and adults who have a strong genetic predisposition as opposed to *P. falciparum* infection and ingestion of quinine in the African population.

In African children, the condition has been reported in stable high transmission areas and during intense transmission of the disease [190]. On the possible causes, identification of myoglobin and haemoglobin in dark urine [185], suggests that aetiology of childhood BWF is diverse. The outcome has also been reported to vary, but seems dependent on aetiology and presence of overlapping

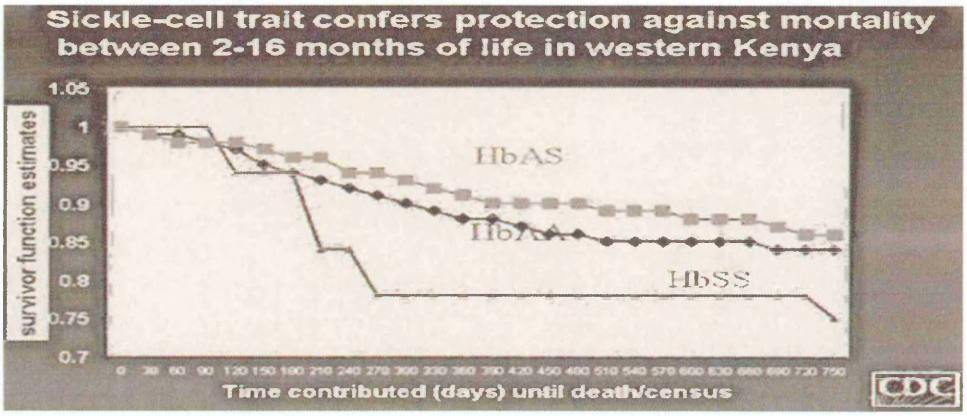
complications. Both myoglobinuria and haemoglobinuria are associated with acute renal failure with poor outcomes [179]. In addition, when cerebral malaria is in the background the outcomes are variable but often poor [185, 194-196].

1.4. The role of red blood cell polymorphism in severe malaria

There seems to be a plausible relationship between malaria and red blood cell (RBC) polymorphism. For instance, there are similarities between geographical distribution of *P. falciparum* malaria and that of RBC polymorphisms in the tropics [197-199]. In East Africa, the common RBC polymorphisms include haemoglobinopathies such as alpha-thalassaemia and sickle cell anaemia (HbSS) [197, 200]. The other categories are enzymopathies, especially G6PD deficiency [198]. These polymorphisms are associated with geographical distribution of malaria, but more importantly, they modify the course and clinical spectrum of the disease depending on dominance of the polymorphic gene in the host [198, 199, 201, 202]. Typically in these polymorphisms the heterozygous states have been associated with resistance or protection to malaria morbidity and mortality [197, 200, 203]. In East Africa, there is a general paucity of data on these conditions but some progress has been registered in Kenya. Williams *et al* found that in the coastal part of Kenya, alpha-thalassaemia in the heterozygous state was protective against malaria OR 0.73 (95%CI 0.57 - 0.94); P=0.013 and similarly, the homozygous state OR 0.57 (95%CI 0.40 - 0.81); P=0.002 [204]. These states were also protective against incident severe anaemia; incident rate ratio (IRR) of 0.33 (95%CI 0.15 - 0,73); P=0.006 [205]. Conversely, the sickle cell gene has also been found to be associated with malaria in several ways. Firstly, heterozygous

polymorphism [sickle cell trait (HbAS)] has been associated with protection against all clinical states of malaria [203] (Figure 1.4).

Figure 1.4. Protection of HbAS against mortality in young children



Source: CDC. Protective effect of sickle cell trait against malaria associated mortality and morbidity. www.cdc.gov/malaria/about/biology/sickle_cell.html

A number of mechanisms for this protection have been put forward including interference with the malaria cycle in the red blood cells and early premature destruction of RBCs by the spleen [204]. The role of immunity may also play part since among African children there seems to be an increased recognition of parasitized red blood cells (pRBCs) in HbAS [204, 206]. On the other hand homozygous sickle cell anaemia (HbSS) gene is known to predispose suffers to severe malaria with poor outcomes [207], especially SMA due to massive haemolysis. However, the low parasitaemia in HbSS patients with malaria disease suggests that HbSS has a protective role, though when infected, patients with HbSS are prone to both severe malaria and poor outcomes [207]. Similarly, in a cohort study in the coastal region of Kenya, Williams *et al* found that in HbAS there was reduction in incidence of both simple malaria IRR 0.49 (95%CI 0.32 -

0.75) P=0.001 and complicated malaria IRR 0.25 (0.16-0.39) P<0.001 respectively [204]. Moreover, they found lower parasite density in both simple malaria P=0.009 and complicated malaria P<0.001 respectively [204]. For the G6PD deficiency there are three common variants with varying distribution across populations. The commonest is the G6PD B, which is the stable form and the next common type is G6PD A+, however, the less common, but often associated with severe manifestations is the G6PD A- allele [199]. The severity of clinical manifestations is inversely proportional to the activity of the enzyme and varies from type B with about 90% to A- with less than 25% activity [199].

1.5. Rare features of childhood severe malaria

Part of the WHO criteria includes some rarely reported clinical manifestations of severe malaria. Both renal failure and disseminated intravascular coagulopathy remain uncommon in children aged less than 5 years [8]. Acute renal failure (ARF) is defined when there is a urine output of <12mL/Kg/24hours and/or in adults a raised plasma creatinine of >3.0mg/dL [8], no creatinine-based definition for children has been considered. Overt ARF is rarely reported in African children with severe malaria, but some studies have shown that ARF in malaria is associated with a poor clinical outcome [179]. Moreover, other manifestations of renal insufficiency are more commonly reported and are associated with a poor outcome [19, 61]. In the AQUAMAT a multicentre study involving 5,426 children across 10 sites in 9 countries, renal insufficiency, defined as an elevated blood urea nitrogen (BUN) >20mg/dL was a poor prognostic feature, as indicated by median BUN among 409 deaths at 24mg/dL compared to 15mg/dL among 3,739 survivors (P<0.001) [61]. In their recent prospective cohort study on acute renal

failure in children with severe malaria in DRC, Kunuanunua *et al* included 378 children in the study (226 male and 152 female) of whom 194 (51.3%) were under 5 years old. Acute renal failure (ARF) defined as (serum creatinine >265μmol/L) was found in 89 (23.6%). Of these 87 (97.8%) with ARF were patients with Blackwater fever (BWF) mainly in children older than 5 years. Their study indicated that quinine use was associated with BWF. Mortality in the ARF group was high 12.6%[179].

Disseminated intravascular coagulopathy (DIC) is a rare manifestation of severe malaria among children but is a contributor to a poor outcome when it does occur [8]. Malaria infection causes depressed platelet levels with subsequent initiation of coagulation pathways but DIC is rare, the fact that suggests that the process may be self-limiting [208]. Since malaria parasites are largely confined to RBCs they do not directly affect body target organs, but its main mechanisms emanate from sequestration of pRBCs in the blood vessels [209, 210]. With the various intravascular processes involving pRBCs including haemolysis of pRBCs, agglutination, rosetting, platelet consumption, circulating *P. falciparum* haemozoin (PfHz), cytokine activation and hypoxia of tissues, several different pathways of the coagulation system are initiated in the process [84, 210]. Initial endothelial activation is followed by activation of platelets, impairment of endothelial lining, and cascading of coagulation pathway [84, 210]. In a study among Malawian children with fatal cerebral malaria (CM), Taylor *et al* found that of the 31 children who died with clinical diagnosis of CM, 24 (77.4%) had autopsy features consistent with CM including vascular parasite sequestration and 75% had vascular damage [72], findings that are consistent with other recent studies on

sequestration, intravascular and perivascular damage [211], and cytoadherence [212]. These may explain the process of thrombus formation in severe malaria. Retinal haemorrhage in CM has been noted to correlate with intra-cerebral bleeding and their occurrence in both the surviving and fatal cases of CM may suggest their role in the disease prognosis [213], since there is a direct correlation between their number and risk of death. The virulence of *P. falciparum* compared to other malaria species may partly be related to its ability to result in thrombocytopaenia in that; initiation of the coagulation cascade occurs in *falciparum* but not in infections caused by other malaria species [214].

1.6. Summary

In conclusion, the clinical features, risk and prognostic factors for *P. falciparum* malaria vary depending on transmission intensity, age and geographical location. In Africa, transmission intensity appears to determine the burden and clinical features of malaria [110]. Studies done in areas with moderate-to-high transmission such as the coastal part of Kenya in the late 1980s to 2005 [8, 215], in Uganda [22, 52], in Malawi [21, 23], in The Gambia [26, 216], in Nigeria [217, 218], in Ghana [219] and Sudan [220], all indicate that severe malaria occurs most commonly among children under 5 years when their immunity to the disease is low and the disease progression is rapid [98, 221]. It is now well understood that there are significant differences in the clinical spectrum of severe malaria with age. Clinical features with poor outcomes among children include impaired consciousness or coma. In an autopsy study of 90 deaths in children with coma, White, confirmed 64 (71.1%) were due to cerebral malaria [63]. The case fatality is high in cerebral malaria ranging from 10 - 41% [24, 222, 223]. The severity of

disease increases with multiple clinical features. Idro *et al* [123] showed that severe anaemia was independently associated with profound coma (P=0.002), and age < 3 years (P=0.001). In addition, these authors reported one out of every three children with severe anaemia had respiratory distress compared to only 15% without (P=0.118). In cerebral malaria mortality is higher in rural settings compared to urban settings [224]. Neurological sequelae are reported in 1 - 10% and are associated with recurrent convulsions, hypoglycaemia and acidosis [37, 39]. Severe malarial anaemia (SMA) is one of the common clinical features of severe malaria in children <5years [113, 117, 132]. Asymptomatic SMA carried with it low mortality of <1% [8] as opposed to the symptomatic SMA with in-hospital deaths as high as 54% [23]; which requires emergency blood transfusion [113]. The outcome is even poorer when associated with acidosis [50]. Other features with poor outcomes include respiratory distress, multiple convulsions and hypoglycaemia [158]. In a multicentre randomised trial (AQUAMAT), the following five predictors of poor outcome were identified: "base deficit (adjusted odds ratio [AOR] 1.12, 95% confidence interval (CI) 1.10–1.13), coma score (AOR 1.40,95% CI 1.34–1.45), convulsions (AOR 1.72, 95% CI 1.30–2.30), BUN (AOR 1.02, 95% CI 1.02–1.03), and chronic illness (AOR 2.12, 95% CI 1.25–3.58)" [61]. In another study, Maitland *et al* in a prospective study on electrolyte disturbances in Kenyan children with severe malaria reported a high case fatality rate 7/9 (78%) in patients with severe hyperkalaemia (potassium >5 mmol/L), OR 21 (2.5 - 163); P=0.009 [158].

1.7. Justification

In Uganda over 90% of the population lives in areas highly endemic for malaria. The burden and clinical spectrum of malaria in the country has partly been described in the South-western, Central and South-eastern parts of the country [22, 24, 65, 123, 225]. However, these studies were done over a decade ago and even within these settings I expect temporal variation because of impact caused by malaria control measures and environmental conditions on the intensity of transmission of malaria. For instance, in the Central region of Uganda, between 2004 and 2006, there was variation in manifestations among children presenting to Mulago hospital with severe malaria [22, 24]. The variations of clinical manifestations between areas with different transmission patterns and geographical locations have been described before [9, 32, 52]. Nonetheless, these data point to the fact that descriptions on manifestations of severe malaria in Africa are incomplete.

To the best of my knowledge, no formal systematic inquiry has been done on the burden and clinical spectrum of severe malaria in Eastern Uganda. Even fewer descriptions, if at all any, have been done on a cryptogenic disease associated with dark urine, severe anaemia and jaundice in Eastern Uganda. The classical triad of haemolysis includes dark urine, severe anaemia and jaundice.

As noted in the literature above, dark urine is not synonymous to haemoglobinuria, even though the earlier research work did not clearly differentiate these two conditions. Earlier data reported BWF based on urine colour, but the classical definition of BWF refers to severe malaria with

haemoglobinuria. There is always haemolysis in *P. falciparum* malaria. The mechanisms for these processes include but may not be limited to: the destruction of non-parasitized red cells probably by complement-mediated mechanism mainly occurring in extravascular compartment. In addition, parasitized red cells are certainly destroyed in the process of schizogony in intravascular space. Massive intravascular haemolysis has been associated with haemoglobinuria.

In some RBC genetic conditions for instance SCA; there is chronic haemolysis irrespective of their malaria infection status. This is mostly extravascular but probably a small proportion of it may be intravascular. In G6PD A- which is the steady state there is no chronic haemolysis; however, if there is a trigger including oxidative stress of any kind, there occurs an acute haemolysis, both intravascular and extravascular. Table 1.3 below summarises the common causes of haemolysis, trigger factors and diagnostic approach in children

Table 1.3 Common causes of haemolysis in children

Condition	Circumstances	Diagnostic Approach
G6PD deficiency	Exposure to a trigger of haemolysis	Test for G6PD activity
Blackwater fever	Relatively rare complication of malaria	Blood slide for malaria parasites
Paroxysmal cold haemoglobinuria	Usually associated with viral infection	Test for Donath-Landsteiner antibody
Mismatched blood transfusion	Usually ABO incompatibility	Repeat crossmatch
Paroxysmal nocturnal haemoglobinuria	Tends to recur	Flow cytometry for CD59
Clostridium welchii septicemia	Burns, severe open trauma, transfusion of contaminated blood	Culture of blood or appropriate patient material

Source: Nathan & Oski's Hematology of Infancy and Childhood, 7th ed, p. 891.

In addition drugs have been known to cause haemolysis. Among antimalarial drugs, the commonly implicated drugs are quinine, chloroquine and primaquine. Amongst antibiotics sulfa containing drugs and quinolones have been known to cause haemolysis in certain condition in children. Analgesics, especially Aspirin have been implicated in causation of haemolysis.

At this stage, it is debawhether one should use BWF as a case description. First and foremost, it is old terminology. Most recent literature use haemoglobinuria in line with the scientific definition of BWF as severe malaria presenting with haemoglobinuria [1]. In addition, at clinical case presentation, it is not possible to differentiate children who present with haemoglobinuria from those who have myoglobinuria or haematuria. Lastly, at research level, it is important to use definitions that allow for specific comparisons between different areas or populations. Therefore, before aetiology of the underlying cause of urine darkness in febrile children is confirmed, I propose the use of the term dark urine syndrome (DUS), a syndrome that will cover dark urine due to haematuria, haemolysis or muscle cell injury in malaria; as was first described in PNG in 2006 [185].

For the children in the Eastern region of Uganda, further understanding of the clinical presentation and possible underlying causes, therefore, is important for immediate case management and in the longer term to understand the effects on the disease epidemiology once effective control and treatment interventions are implemented. In addition, it will enhance the knowledge base on childhood severe malaria in Uganda.

1.8. Specific aims and hypotheses

In this thesis I aimed at providing a comprehensive description of children with severe illness that incorporates severe malaria in an area of intense malaria transmission, which will be of relevance to researchers, clinicians and policy makers. The major aims for my research project have been succinctly presented in each chapter, but in brief:

In Chapter 1, I aimed at presenting background literature on severe malaria and BWF, while identifying research gaps that my project addressed in the context of childhood severe malaria and backwater fever in Eastern Uganda.

In Chapter 2, I present the materials and methods used in the paediatric ward surveillance study (WSS), severe malaria surveillance (SMS) and prospective dark urine epidemiological study (PRODUES). It therefore covers aspects of the study design, data collection, analyses and management. In addition, I describe the laboratory procedures done at Mbale Regional Referral Hospital (Mbale RRH) and those done at KEMRI Wellcome Trust Research Laboratory in Kilifi, Kenya.

In Chapter 3, I aimed at, for the first time, to formally describe the patterns of paediatric admissions, their clinical features, burden of malaria and blackwater fever in relation to other admissions to the paediatric acute care unit (PACU) at the Mbale RRH. In this chapter, I provided a detailed account on the burden of disease by profiling the clinical manifestations in children <1 month and those > 1 months; clinical admission diagnoses and seasonality of these illnesses. The significance of this part of my study project was that it provided entry criteria for identification of cases with severe malaria and those with DUS. In addition it

provided a baseline for reference and interpretation of analyses on these two conditions.

In Chapter 4, I aimed at describing the spectrum of the clinical manifestations of childhood severe malaria in Eastern Uganda and to identify the factors, which might have associations with the development of complications and mortality in childhood severe malaria in this region. Being the first time this description has been done at Mbale RRH and the region, it sets a baseline and a reference point for temporal trends in the subsequent descriptions.

In Chapter 5, I aimed at describing the prevalence of DUS in children admitted to hospital with severe febrile illness in 6 centres in East Africa. In addition, I compared the clinical features in children presenting with DUS with those without, in order to get a better understanding of the clinical spectrum and complications of DUS. Furthermore, I identified potential risk factors, which might have associations with the development of blackwater fever and poor outcomes among children presenting with BWF. I also used these findings to postulate some hypotheses and further research questions for a prospective study.

In Chapter 6, I based on a set of hypotheses in Chapter 5 in order to describe the aetiology, complications and outcome of DUS in a prospective study. In this chapter, therefore, I identified all children presenting to the PACU at Mbale RRH with suspected episode of DUS and recruited them to the PRODUES. I described the clinical features of DUS at admission and frequency of complications of jaundice and severe anaemia both individually and as a triad. In addition, I described the outcomes for patients with dark urine in Eastern Uganda.

Furthermore, since most literature correlates malaria with BWF, I compared children with malaria slide positive and those with malaria slide negative at admission and noted clinical similarities and differences. Finally, I described the community distribution of BWF in Eastern Uganda.

In Chapter 7, I discussed the findings in my study project, noted limitations and identified areas requiring further research, some of which I hope to take up as a means of furthering my understanding on DUS in Eastern Uganda.

CHAPTER 2: Materials and Methods

2.0. Introduction

In this chapter I will describe the research materials, methods, techniques and data analyses that I used in the studies presented for my thesis. As described in the previous chapter, my work aimed at addressing research questions and hypotheses relating to childhood severe malaria and the dark urine syndrome in Eastern Uganda. I identified and developed my research questions during the course of the FEAST trial (ISRCTN 69856593) [159].

2.1. Literature search

2.1.1. Participants

The literature review for this study project was done taking into account various aspects, for instance, studies that included children in published malaria studies. In addition, adult participants for comparison of severe malaria spectrum were considered. Furthermore, geographical location was taken into account. I primarily concentrated on literature on malaria from the SSA. Studies from the SEA were used for drawing comparisons of spectra of malaria in between SEA and SSA, highlighting the uniqueness of my research project.

2.1.2. Interventions

Publications that evaluated various interventions in malaria were also used especially interventions on severe malaria and their outcomes.

2.1.3. Outcomes

My review also emphasized studies that included mortality as outcome since

severity of malaria is measured by the contribution of clinical features to mortality.

2.1.4. Study designs

I considered both descriptive epidemiological studies and clinical trials. However, for eastern Uganda, since there were no published studies on dark urine syndrome from this area, I included observations by leading clinicians in the region to bring into context the local magnitude of the problem.

2.1.5. Search strategy

The search strategy for published studies included an initial limited search of the WHO publications on childhood severe malaria on www.who.int/en; from where I identified key words for this study including: child, severe malaria, sub-Saharan Africa (SSA), South East Asia (SEA), prostration, coma, impaired consciousness, respiratory distress, severe anaemia, jaundice, hypoglycaemia, multiple convulsions, abnormal spontaneous bleeding, hyperparasitaemia, renal impairment, black water fever, haemoglobinuria, circulatory collapse, pulmonary oedema, adult and mortality. I then considered two publications that systematically reviewed severe malaria in children, before and after 1990. This year corresponded to the introduction of the first broad WHO criteria for surveillance of severe malaria before it was updated in the year 2000 [25]. Therefore the literature before and after 1990 gave me both the historical background of the clinical spectrum of severe malaria as well as its scope in these two eras. Using the key words identified above I analysis the text words contained in the title and abstract for publications on severe malaria on each of the clinical

features in the updated WHO criteria [1]. I therefore chose and included studies that described severe malaria spectrum or individual clinical features of the spectrum. I accessed and used the full publications in PDF from the following databases PUBMED, HINARI or Google Scholar.

For the dark urine syndrome in Eastern Uganda, I referred to the local hospital reports and observations Engoru and I had made on this syndrome over the last decade. Lastly, a reference list of all articles and reports used was made and appended to my thesis. For quality purposes, I shared my references with my supervisors from time to time when they reviewed the various chapters and during the supervisory meetings.

2.2. Scope of the study project

My work for this thesis, covers four interrelated projects including: (1) a surveillance study conducted on the paediatric admissions at Mbale Regional Referral Hospital (Mbale RRH), here referred to as the ward surveillance study (WSS), which aimed at describing the patterns of admission in terms of clinical childhood diseases; (2) severe malaria surveillance (SMS), a sub study of (1: WSS) aimed at describing the burden and clinical characteristics of severe malaria among children presenting to Mbale RRH; and (3) studies on dark urine phenomenon further subdivided into (i) a retrospective study on dark urine epidemiological study (REDUES), and (ii) a prospective dark urine epidemiological study (PRODUES) which was also a nested sub-study of (1: WSS). Studies 3 (i) and (ii) were complementary to each other, and were aimed at describing the burden, clinical characteristics and possible causes of the dark urine syndrome among children presenting in Eastern Uganda.

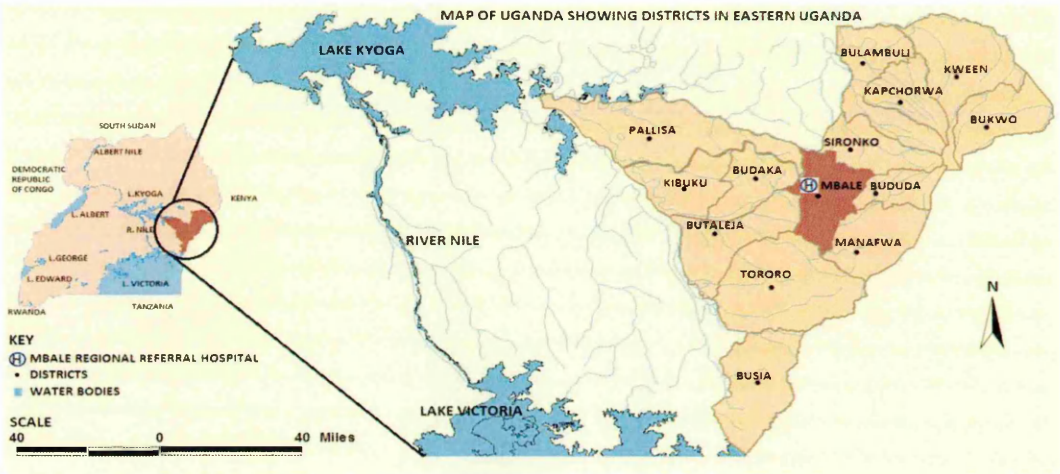
For the purpose of presentation of this thesis, all the materials and methods I used in the prospective studies have been described in this chapter, although each chapter has a brief section on methods. Moreover, I have covered those for the retrospective study in Chapter 5.

2.3. Study site

Mbale Regional Referral Hospital (Mbale RRH) is located at the heart of Mbale Municipal Council, 214km to the east of the capital city Kampala. It is the main referral hospital in the Elgon zone in Eastern Uganda, a geographical area that borders the western part of Kenya (Figure 2.1). The hospital is situated at an altitude of 1,140m and the elevation of its catchment area ranges from 980 to 1,800m above sea level. A majority (over 70%) of the inhabitants in this area are of the Bantu ethnicity and >90% live as a rural agrarian community with <10% of the population living in urban areas. The Mbale RRH is a government run, not-for-profit, charge-free, 470-bed hospital with 4 major general specialties: surgery, internal medicine, obstetrics and gynaecology, and paediatrics. The hospital serves a catchment population of approximately 4.5 million people in the 14 districts of Budaka, Bududa, Bulambuli, Butaleja, Bukwo, Busia, Kapchorwa, Kibuku, Kwen, Manafwa, Mbale, Pallisa, Sironko and Tororo (Figure 2.1). Despite its referral status, Mbale RRH provides mainly primary care, although the hospital receives a number of referrals from the district hospitals and lower health units in the region. The region has favourable environmental attributes for breeding, multiplication and efficiency of anopheles mosquito, which include the elevation of the area (980 - 1,800M), predicable rain pattern (two rain peaks seasons) and suitable ambient temperatures (low 15^{O}C and high 31^{O}C) [226-229]. Whereas, the

annual entomological inoculation rate (EIR) in Mbale has not been documented, the EIRs of the surrounding areas with similar altitude, rain pattern and temperature are higher. For instance, Tororo district to the south and one of the districts in the hospital's catchment area has a recent EIR of 125 (95%CI: 72.2 - 183.0) in 2013 [229], but previously in 2006, it was 397 [227]; indicating perennial stable transmission. Moreover, 240km northwest of Mbale is Apac with EIR of 1,586 [227], the highest EIR ever reported in the world. These findings suggest that malaria transmission in Mbale and its catchment areas is stable, intense and perennial.

Figure 2.1. A map of Uganda with inset showing the catchment area for Mbale RRH in the Eastern Uganda



Using ArcGIS (WWW.ARCGIS.COM/EN/MAPPING), the ArcGIS map of Uganda was accessed as a primary user interface. in addition, a base map, data layers and legend were accessed. Furthermore, exposition of the 14 districts with their respective boundaries (cinnamon colour and Mbale district in brown) which form the catchment area for Mbale RRH (H) at the centre of Mbale district. Finally, water bodies including rivers, tributaries and lakes in blue colour were included.

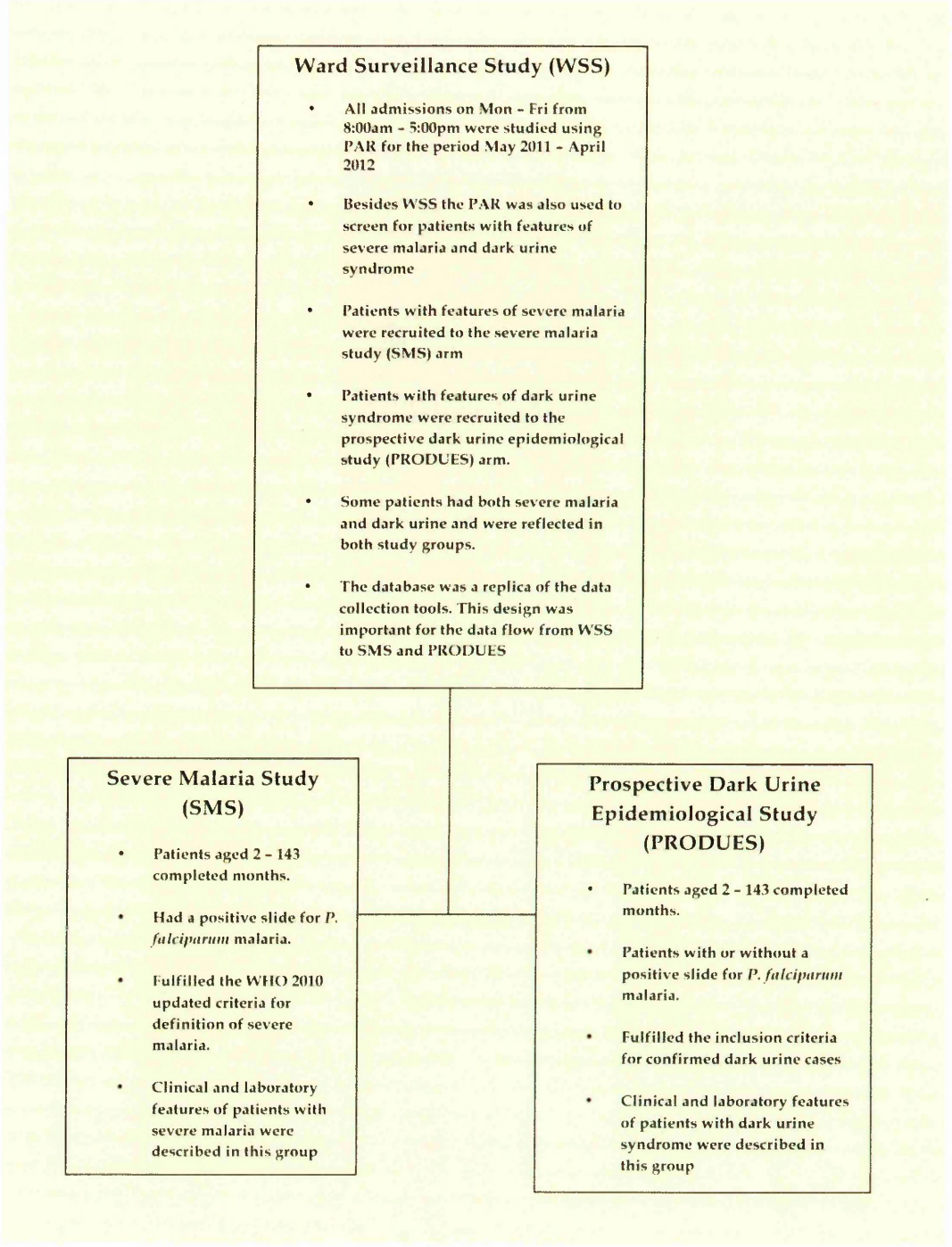
The Department of Paediatrics and Child Health at Mbale RRH has two consultants, one specialist, two medical officers and 17 nurses. My epidemiological studies were conducted on the Paediatric Acute Care Unit (PACU) at Mbale RRH, which is within the department of Paediatrics. All acutely sick children are admitted to the department through the PACU. The total annual admissions to the PACU have varied over time. For instance, in the last 5 years alone, the hospital records indicated that the annual patients load in the financial year (July/June) were 13,000 in 2007/2008 and 24,000 in 2012/2013 (Mbale hospital records 2010 - unpublished). From January 2009 to January 2011, the hospital conducted the FEAST (Fluid Expansion As Supportive Therapy) Trial, which resulted in a marked general improvement in paediatric services and care, a factor that may have influenced patient numbers presenting to the unit. Moreover, my pre-study summary data from the hospital records office in 2010 at Mbale RRH indicated that ~20% of all admissions to the PACU were for children with critical and often life threatening illnesses (Mbale hospital records 2010 - unpublished).

2.4. Ward surveillance study (WSS)

This was a prospective descriptive paediatric admissions ward surveillance study (WSS) with a view of describing the clinical features, disease burden, outcome and their temporal relationships with seasons among children admitted to the PACU. The study team comprised of 5 trained clinicians, who reported to the PACU daily on Monday to Friday to collect data using a specifically adapted and tailored paediatric admissions record (PAR) (PAR; see appendix: data collection tools). Data were collected on all children presenting to PACU during the 8-hour day

service shift of 8:00am to 5:00pm for the one year period 1st May 2011 to 30th April 2012. I identified all the data elements to be collected in order to meet the objectives of the ward surveillance as specified in the protocol and included them in the PAR. The proforma had two pages; on the first page were sections to gather information about patient identifiers, demographic characteristics and medical history. The second page contained sections on physical examination, laboratory investigations, diagnoses, treatment, status at discharge and initials of the study clinician. The PAR also included questions for the clinical screening of severe malaria and dark urine syndrome because the WSS was an entry point for identification of cases for these sub studies. The ethical considerations for the WSS have been covered in chapter 3. Any patient who qualified for either severe malaria surveillance (SMS) or prospective dark urine epidemiological studies (PRODUES) was invited to these studies as described in section 2.3 and section 2.4 respectively. This linkage also informed a logical database design in order to minimize potential errors when transcribing data. This also provided a smooth flow of data from WSS to either SMS or PRODUES (Figure 2.2).

Figure 2.2. Study flow chart for the surveillance



Both the SMS and PRODUES were nested into the WSS

The database was build to allow easy flow of data through the planned study flow structure. The WHO 2010 criteria were used for clinical surveillance of severe malaria, while the parent/guardian confirmation of dark urine was used as entry criteria for the surveillance of dark urine syndrome.

The responses in the PAR proforma were of two types depending on the questions: the first, were binary "yes" or "no" answers which formed 90% of the response type in the tool while the remaining 10% were free text responses especially on alternative diagnoses and sections of systematic examination demanding the study clinician to document additional notes on the extra findings (See appendix: data collection tools). This design made it easy to complete the form. I collected raw data wherever possible rather than calculated data (e.g. for age: the raw form is date of birth while the calculated form is child's age at admission) this was done to improve on the accuracy and easy validation of data. Moreover, each question was constructed to oblige a definite response in order to improve precision of the responses and minimize variability in the database. Data regarding basic laboratory tests conducted, including malaria blood slide examination, haemoglobin (Hb) estimation, and where indicated, the results of cerebral spinal fluid (CSF) analysis and urinalysis, were transcribed from the laboratory forms and report book (source documents) and recorded into the PAR proforma. Similarly, a treatment plan for each patient's condition was made on the PAR-proforma according to the Uganda National Treatment Guidelines 2010.

The following samples were collected for routine tests during the paediatric ward surveillance: a small volume of blood [a drop of blood approximately 0.1mls] on a

slide for malaria test, 0.1mls blood for Hb estimation (HemoCue) for all patients who were clinically pale, 1.0mls CSF for all patients with either coma or an index convulsion. In addition, a small volume of blood (0.1mls) was collected for random blood sugar and 0.1mls for lactate level estimation from all children with impaired consciousness and respiratory distress. Furthermore, a small blood sample as mentioned above was collected for lactate level estimation for children with features of severe malaria. For children suspected to have a urinary tract infection, 2mls of urine was collected and sent to the laboratory for urinalysis. All other tests and investigations that were necessary for making definitive diagnoses, further management and follow up of patients were ordered at the discretion of the attending clinician. The hospital has in place an opt-in policy for routine HIV testing which applied to all patients reporting for care at the PACU during my study period.

2.5. Severe malaria surveillance (SMS)

As part of the WSS, children were screened for clinical features of severe malaria and those found eligible were recruited to the SMS. Eligible patients were identified on the following criteria: recorded fever or a history of fever in the current illness plus any one or more of the WHO 2010 updated criteria indicated on Table 2.1 below. These are fairly broad criteria since they are applicable to adults and children across the range of transmission intensities and have previously been used, for instance, in a recent descriptive study of severe malaria in Kampala, Uganda [10] and based on previous studies in Tanzania [58], The Gambia [11] and Kenya [8]. Finally, any patient with these clinical features were

confirmed as a case of severe malaria if their peripheral blood smear was positive for asexual forms of *P. falciparum*.

Table 2.1. WHO 2010 clinical definition of severe malaria [1].

The WHO Clinical Features for Severe Malaria in Adults and Children
Clinical features
• Impaired consciousness or unrousable coma
• Prostration, i.e. generalized weakness so that the patient is unable walk or sit up without assistance
• Failure to feed
• Multiple convulsions - more than two episodes in 24 h
• Deep breathing, respiratory distress (acidotic breathing)
• Circulatory collapse or shock, systolic blood pressure <70mmHg in adults and <50mmHg in children
• Clinical jaundice plus evidence of other vital organ dysfunction
• Haemoglobinuria
• Abnormal spontaneous bleeding
• Pulmonary oedema (radiological)
Laboratory findings
• Hypoglycaemia (blood glucose <2.2mmol/L or <40 mg/dL)
• Metabolic acidosis (plasma bicarbonate <15mmol/L)
• Severe normocytic anaemia (Hb <5g/dL, packed cell volume <15%)
• Haemoglobinuria
• Hyperparasitaemia (>2%/100 000/μL in low intensity transmission areas or >5% or 250 000/μL in areas of high stable malaria transmission intensity)
• Hyperlactataemia (lactate >5 mmol/L)
• Renal impairment (serum creatinine >265 μmol/L)

Source: WHO Guidelines for Treatment of malaria. Second Edition 2010, Geneva.

In my study, I clarified some of the definitions for features in the table above for ease of their application. Coma was defined as an unrousable state with a

corresponding Blantyre Coma Scale ≤2 for which no other cause other than malaria could be identified. This was a similar definition used by earlier researchers on severe childhood malaria [8, 31, 96]. To exclude intracranial infectious causes coma, a lumbar puncture (LP) was done. The clinical standard operation procedures of LP before during and after were followed. The procedure involved inserting a hollow needle into the subarachnoid space in the lumbar area (lower back) at L4/5 of the spinal column. The subarachnoid space is the canal in the spinal column that carries cerebrospinal fluid (CSF) between the brain and the spinal cord. During the procedure we noted the opening pressure, flow and colour of CSF. At microscopy, a gram stain was done. In addition the CSF sample was analysed for white blood cell counts, sugar and protein levels. Only patients with normal CSF qualified the definition of malarial coma.

I considered spontaneous bleeding as physically un-induced and irrepressible bleeding from at least 2 non-traumatized sites in a patient with severe malaria without previous history of abnormal bleeding. Haemoglobinuria was macroscopically detected tea or Coca-Cola colored urine. This was similar to descriptions of haemoglobinuria in DRC [179, 190], Nigeria [180] and Burundi [183], while clinical jaundice was yellowing of mucous membranes noted in sufficient daylight. Finally, I defined severe malaria deaths as any in-hospital fatality in a child aged 2 - 143 completed months presenting to hospital and at a time of death had history of fever, confirmed *P. falciparum* and clinical features of severe malaria [1]. Furthermore, hyperpyrexia was defined as axillary temperature ≥ 40.0^{o}C.

Patients with features of severity and eligible for the SMS were invited to participate in the SMS after informed consent was sought from the patient's parent or guardian (section 2.3). I designed an appropriate case report form (CRF) for this surveillance study (Appendix - data collection tools). Briefly, the sections of this CRF were organized to logically capture data on: socio-demographic features, clinical features (symptoms and signs), laboratory data, treatment and outcome.

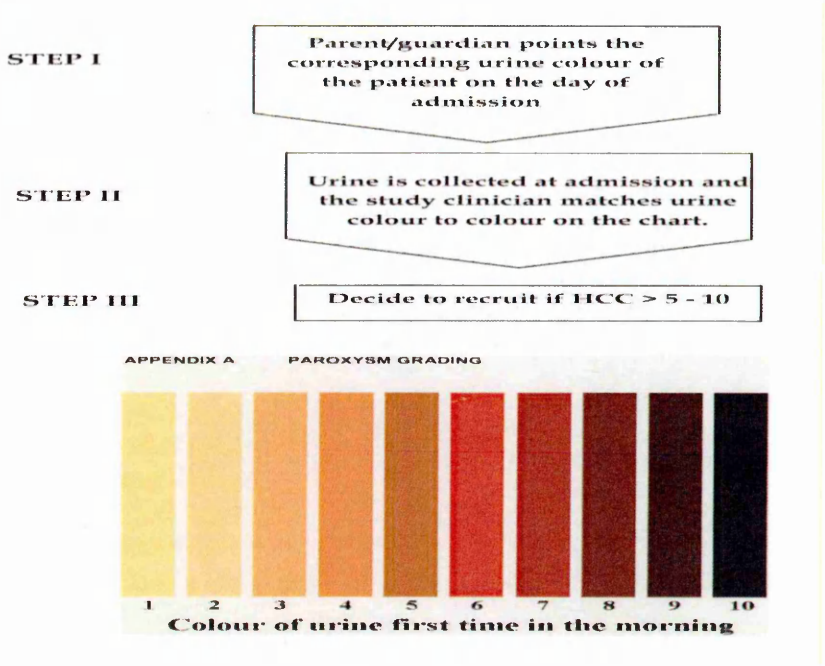
Blood samples for the SMS were collected for parasitological microscopy as well as estimation of lactate, haemoglobin and random blood sugar levels. In addition, a small volume of blood was collected for additional tests including: 2mls of whole blood for complete blood counts (CBC), 0.1mls for quality controlled blood slide, 0.5mls whole blood EDTA for genotyping. The blood for genetic assays was stored at the adjacent JCRC laboratories, Mbale at -80^{O}C, where the freezers' temperatures were monitored daily and logged. In addition these freezers had automated alarms to alert the laboratory team whenever the temperature was off the range. Furthermore, during transportation, all the samples were packed in dry ice throughout the journey to WTRP Kilifi, Kenya, from where genotyping was done.

2.6. Prospective dark urine epidemiological study (PRODUES)

Patients enrolled to the WSS with a history of passing dark urine during the course of their current illness were eligible for further evaluation before inclusion in the prospective dark urine epidemiological study (PRODUES). Eligible patients were initially assessed on the basis of history and classified as suspected cases of dark urine. In addition, an inquiry about the child passing dark urine, defined as

Coca-Cola or tea coloured urine on the day of admission was made. Furthermore, asking the parent/guardian to indicate the grade (by pointing) at the colour against the Hammersmith Colour Chart (HCC) (Figure 2.3) qualitatively assessed the urine colour. I adopted the use of HCC as an additional objective criterion for differentiating suspected from probable or confirmed cases of dark urine syndrome. The HCC had 10 colour codes ranging from mild yellow (colour code 1) to black (colour code 10). At admission, the patient/guardian was first asked to recall and matched the colour of urine passed by their child on the day of admission to a colour on the chart. These patients were identified as suspected PRODUES cases. Urine was collected from the child using paediatric urine collection bags before it was transferred into the urine collection bottle. The study clinician then matched the urine colour to the corresponding score on the HCC scale. Children with clinician-witnessed urine corresponding to HCC ≥5 were confirmed to have dark urine syndrome. There were children whose parents or guardians indicated urine color on HCC ≥5 but were not able to pass urine. In these cases their clinical features were noted but their data were not included in the final analysis because the data verification of the urine HCC grade were excluded. For easy access and efficient use of the HCC, charts were displayed both in the patient screening area and in the ward, and pocket-sized copies were also issued to the study clinicians.

Figure 2.3. Hammersmith urine Colour Chart (HCC) and how it was used in this study.



The criteria used relied on the parent's ability to identify the correct colour of the patient's darkest level of urine prior to admission and the child's ability to pass urine within the 3 hours of admission.

Following study enrollment, the PRODUES CRF (see appendix: data collection tools) was completed. It was designed and organized to capture data on: sociodemographic features, clinical features (symptoms and signs), laboratory data, treatment and outcomes.

Two types of samples were collected from the PRODUES study participants. Urine was collected in 15 - 20mL volume and three aliquots were obtained from these. The first aliquot of 2 - 4mL was used at bedside for confirmatory HCC reading and urine Multisick testing. The second aliquot of 4mL was sent to the hospital clinical laboratory where microscopy for *S. haematobium* was done on all collected urine samples. The third aliquot of 4 – 10mL was stored at -80^{O}C and shipped to

KWTRP, Kilifi Kenya for haemoglobin and myoglobin assays. Blood was collected as follows: at the WSS blood for blood slide, lactate, haemoglobin and random blood sugar estimation was collected as described in section 2.2. Five millilitre samples of blood were collected from patients at the point of admission, which were aliquoted into two 0.5mL EDTA tubes and the rest was put in one 5mL heparin tube. One EDTA tube was used for full blood count (FBC), blood grouping, Coombs test and malaria parasite determination. The second EDTA tube was stored for later DNA extraction and molecular detection of red cell genetic polymorphisms in Kilifi, Kenya. The heparin tube was centrifuged at the study laboratory before plasma was aspirated into two equal aliquots using a sterile pipette and stored at -80^{O}C for later biochemical testing including: haptoglobin, methaemoglobin, myoglobin, haemoglobin and immunology assays (HRP-2 ELISA).

At follow up (convalescent patients), we collected one EDTA of 0.5mL of blood on which we did blood slide for malaria parasites, reticulocyte count, G6PD activity and complete blood counts (CBC). In addition, about 4mL of urine were collected from which three aliquots were prepared. Two of the aliquots each containing 1.5mL were stored for urine haemoglobin and myoglobin tests in Kilifi laboratory. The third was used for dipstick tests and microscopy for *Schistosoma haematobium* at Mbale RRH. The diagnostic procedure for *S. haematobium* involved pipetting about 5mL of urine to a falcon tube and the aliquot was span in a centrifuge at 3000rpm for 3min. The supernatant was discarded into a discard jar. A drop of the deposit was then put on a microscope slide and covered with a coverslip. The preparation was examined using X10 and X40 looking for presence of WBCs, RBCs, casts,

yeasts and parasites. The report was made as either "No schistosoma seen" or "Schistosoma seen". In case of the latter quantification as +, ++, +++ or ++++ would be done depending on the number seen.

The Coombs test procedure involved placing 100µl of blood in a clean test tube, then add 1mL of 0.9% sodium chloride to wash the cells and spin for 1min at 1000rpm using a centrifuge. The supernatant was aspirated and another 1mL of 0.9% sodium chloride was added to wash the cells again and spin for another 1min at 1000rpm and a supernatant removed as before. From the resulting deposit, a 3% cell suspension was made on which a drop of Anti-Human Globulin (AHG) was added and mixed. This was followed by incubation for 5min at room temperature, spinning the mixture at 1000rpm for 1min. It was then observed for any clumping. The result is recorded as positive if there is visible clumping or negative if there is none.

2.7. Studies to explore risk factors for dark urine

2.7.1. Case-control study

Inherited red cell disorders as potential risk factors associated with clinical dark urine were explored in a case-control study. Cases included patients on whom dark urine was clinically confirmed, aged 2 - 143 completed months and were resident in one of the 14 districts served by Mbale RRH. I excluded patients who were aged <2 or >143 months, those coming from outside of Mbale RRH catchment area, patients with physical trauma (burns, falls and road traffic injuries) and renal disease. Controls were newborn babies born at the maternity unit of Mbale RRH. Recruiting both the cases and controls from the same hospital

maximized the prospect of cases (with dark urine) and controls (newborns) coming from similar communities. In this case-control, the units of observation and analysis were individuals among the cases and controls. The cases were 268 with confirmed case definition of dark urine while the unmatched controls were 496 newborns, making a ratio of cases to controls of 1:1.85. Blood from cases and controls was genotyped for G6PD deficiency, the mutation conferring sickle cell trait (HbAS) and sickle cell anaemia (HbSS) and alpha-thalassaemia. The frequency of these inherited disorders was calculated in both the cases and controls, their Odd's ratios and *P-values* were computed as a measure of association of genes of interest with a likelihood of having dark urine. The results were summarized and reported in a table.

2.7.2. Community GPS survey

As a means of understanding the community distribution of dark urine syndrome and proximity of their residence to environmental risk factors for malaria and other infectious diseases, the distribution of cases was mapped by means of geographical position system (GPS). This generated geospatial coordinates with longitudes, latitudes and altitudes within 3 metres of precision of PRODUES patient houses, health facilities, and physical features including mountains, rivers and swamps.

The aim was to get some insight to the understanding of environmental risks for breeding of *anopheles* mosquitos in the community. In addition, I wanted to understand whether there was potential delay by these cases accessing medical services and therefore, assessed their proximity to established health units in the

community. By locating the residential places of the patients within 3metres of accuracy, we were able to estimate the distance between their residences and Mbale Regional Referral Hospital and also from the nearest health unit. This information is useful in making inferences on the contribution distance to access healthcare services. In addition, there has been no geospatial data relating in possible association between physical features including mountains, lakes, rives, swamps, other water bodies dark urine syndrome. Furthermore, there has been no information on the contribution of road infrastructure to the access to health services in Eastern Uganda.

Using the patient locator information, survey plans were made so that the study participants in the same location or in nearby villages were surveyed together to allow for systematic and easy survey procedures. Service availability mapping (SAM) to locate all the health units in the districts where the patients with dark urine came from was done. This method is similar to one previously described by Chamla *et al* in their Geographical Information Systems study of health facilities in Gulu, Uganda [230].

2.8. Laboratory procedures and analyses

The bedside tests, haematological, parasitological and immunological assays requiring fresh samples were performed at Mbale RRH. In addition, molecular assays were performed at the KEMRI-Wellcome Trust Research Programme (KWTRP) in Kilifi, Kenya using stored samples.

2.8.1. Bedside assays

2.8.1.1. Haemoglobin concentrations

Before my study project, the hospital had a number of methods of routine Hb estimation. The Sahli method was still in place but was being replaced by the Calorimetric method. On the other hand, the clinicians were using clinical assessments of pallor. Though clinical assessment of pallor of mucous membranes was at the time used for triage purposes, in instances where bedside or laboratory methods were not operational, clinical method was used to decide on whether or not a child needed a blood transfusion. This was the time when we had just completed the FEAST study where Hb estimation among study participants was done by HemoCue method. We therefore did a quick assessment of all the in-house assessment of Hb so as to decide the most useful method or a combination of methods in our settings. I was the principal investigator of this study. Briefly, all patients reporting were triaged on arrival. Patients found clinically pale were recruited into the study following informed consent. The admitting nurse completed a basic paediatric admission sheet on all children entering the study at admission. This nurse indicated the degree of pallor (none, moderate, severe) and whether she felt on this assessment that the child may require transfusion. The admitting clinician (Clinical officer or medical officer) completed a similar patient admission record and in addition; an assessment of pallor and whether or not they felt the clinical severity of pallor would warrant transfusion (i.e. on clinical

grounds they think the Hb level is <4g/dl or less than 5g/dl with other features). A second clinician also assessed the child for signs of pallor and clinical indication for transfusion so as to assess the inter observer variation. All these clinical assessments were conducted prior to, and thus without knowledge of Hb estimation by HemoCue which was a gold standard. Following these clinical assessments haemoglobin level was assessed from a single sample of blood by the five-study methods (HemoCue, Haemoglobin Colour Scale (HCS), Calorimetric Sahli and clinical). Treatment decisions were made on the result from Sahli or Calorimetric methods, as was the current practice in this hospital, in order to comply wills national guidelines on standard of care. Results from Sahli' or Calorimetric method were given to the treating doctor in order to best manage the patient. There was no delay in receiving treatment due to the study. The different Hb results were logged onto dedicated study sheets alongside child's hospital number. I analysed these data and found that clinical assessments by well trained clinicians provided a good indicator for children who would require blood transfusion. It was also cheap and readily available method. Furthermore, it was sustainable since it did not depend on availability of electricity, equipment and supplies (Olupot-Olupot, unpublished manuscript). In my study therefore, I decided to use clinical method for identification of children with pallor and confirm the Hb level using the HemoCue method.

The final reported Hb results for my thesis were estimated using the HemoCue Hb 301 system, Ängelholm Sweden (Figure 2.4). This system has high accuracy, is cost effectiveness and practical in any environment compared to traditional Hb estimation methods [231]. Basically, it is a portable quantitative point-of-care test

that measures Hb concentration in samples of whole blood using a specially designed analyzer and microcuvettes. The microcuvette serves as both a pipette and measuring cuvette. A blood sample of approximately 10µL was drawn into the cavity by capillary action. The filled microcuvette was inserted into the HemoCue Hb 301 Analyzer. The measurement takes place in the analyzer, which measures the absorbance of whole blood at a Hb/HbO_2 isobestic point. The analyzer measures at two wavelengths (506 and 880 nm) in order to compensate for turbidity. The Hb/HbO_2 isobestic point at the wavelength 506 nm is defined as the optimal wavelength for determination of the hemoglobin concentration with the HemoCue Hb 301 system. The system is factory calibrated and needs no further calibration. The measurement range is 0 - 25.0g/dL and results are available in 10 seconds.

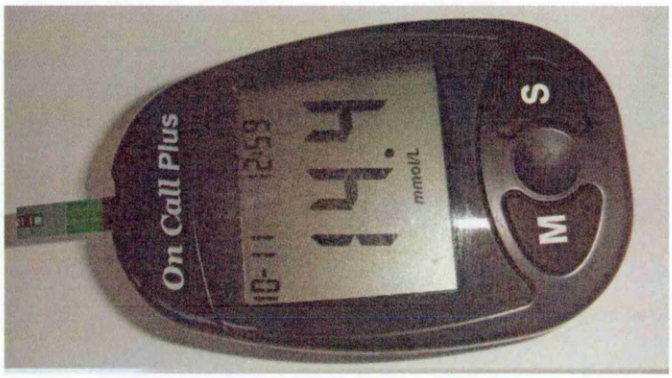
Figure 2.4. Picture of HemoCue Hb 301 system used in Mbale RRH

This is a point of care portable system for measuring the level of Hb in whole blood. The system is calibrated against the hemiglobincyanide (HiCN) method, the international method for determining Hb concentration in blood.

2.8.1.2. Blood sugar estimation

Random blood sugar (glucose) measurements were conducted using *On Call Plus* [ACON Laboratories, San Diego, USA (Figure 2.5)]. This is a biosensor that uses 1μL of plasma drawn from a finger prick by end-fill capillary action and provides the results in 10seconds. This equipment is capable of reading the level of blood sugar in the range 0.6 - 33.0mmol/L, this makes it ideal in emergency settings where decisions for instance to use 25% dextrose or anticonvulsant or both in actively convulsing children are needed in a timely fashion.

Figure 2.5. Picture of the *on call plus*, a glucometer used in Mbale RRH



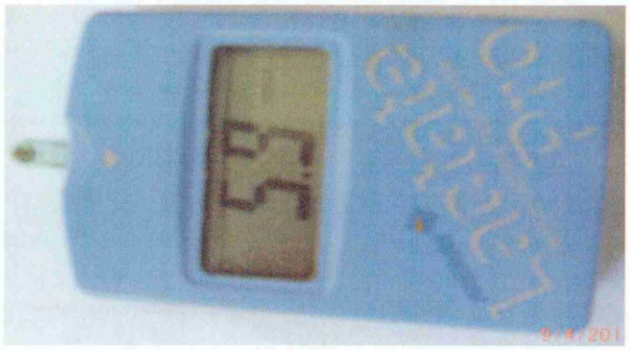
The system has 300-test memory with date and time. Calibration is done by use of a code chip and the measurement is given in mmol/L.

2.8.1.3. Lactate levels estimation

Lactate was estimated using hand held device, Lactate Pro, ARKRAY Factory, Shiga, Japan (Figure 2.6). This is a hand-held, palm sized, lightweight (50g) portable lactate analyser. It uses a small blood sample obtained from a finger prick equivalent to 5μL for analysis. There is an alarm indication once the strip is adequately filled with blood. This equipment has a check strip to confirm that the analyser is operating correctly, a calibration strip to match the function number

displayed with that on the rear of the box of the test strips, and the test strip for blood sample analysis. The principle of lactate analysis used by this equipment is that the lactate in the sample reacts with potassium ferricyanide and lactate oxidase forming potassium ferrocyanide and pyruvate. The ferrocyanide is oxidized on application of a given voltage and releases electrons creating a current, which is directly proportional to the lactate concentration in the blood sample. The result is displayed in 60seconds. The range of lactate values measured by this analyser is 0.8-23.3mmol/L.

Figure 2.6. Picture of lactate pro, a lactate analyser used in Mbale RRH



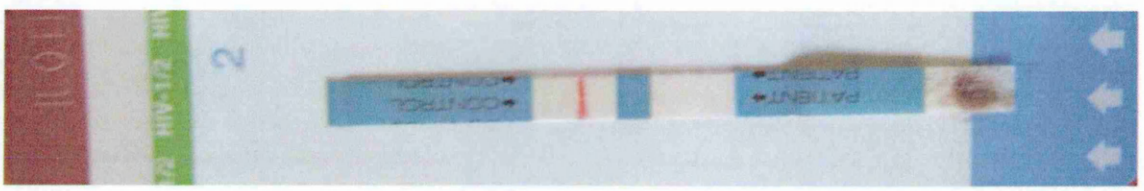
The lactate Pro measures blood lactate in the range 0.8 – 23.3mmol/L.

2.8.1.4. HIV antibody testing

Testing for HIV antibody status at admission was not mandatory for my surveillance, but Mbale RRH implements the opt-in policy for HIV testing. Therefore, the HIV results from the routine HIV testing programme were shared with my study for all admissions at the hospital where HIV was assayed using Determine HIV-1/2 strips, (Alere Medical Co. Ltd, www.determinetest.com) (Figure 2.7). This is an HIV-1/2 Antigen and antibody immunochromatographic

test for the simultaneous and separate qualitative detection of free HIV-1 p24 antigen and of antibodies to HIV-1 and HIV-2. The test device is a laminated strip that consists of a sample pad containing monoclonal biotinylated anti-HIV-1 p24 antibody, a conjugate pad containing monoclonal anti-HIV-1 p24 antibody-colloidal selenium and HIV-1 and HIV-2 recombinant antigen-colloidal selenium, and a nitrocellulose membrane with an immobilized mixture of recombinant and synthetic peptide HIV-1 and HIV-2 antigens in the lower test area, immobilized streptavidin in the upper test area, and an immobilized mixture of anti-HIV-1 antibodies, HIV-1/2 antigens, and HIV-1 p24 recombinant antigen and anti-HIV-1 p24 monoclonal antibody in the control area.

Figure 2.7. HIV negative result on HIV-1/2 antigen-antibody test strip



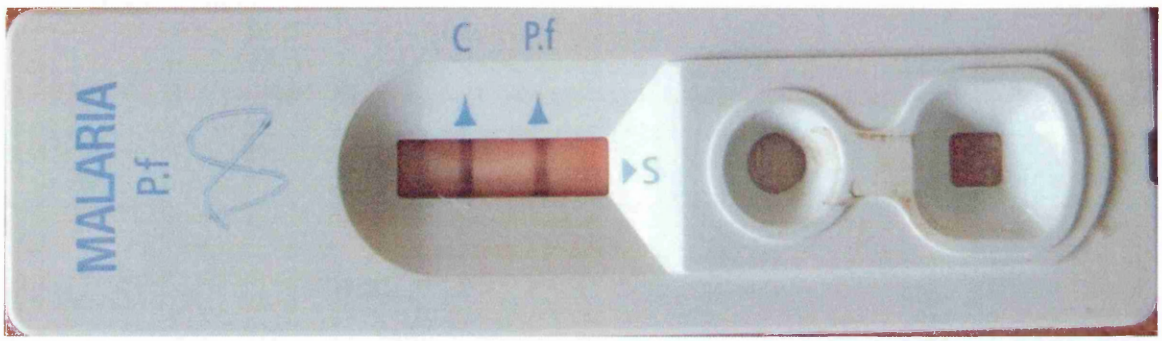
The HIV-1/2 antigen-antibody test strip indicating a negative result

2.8.1.5. Test for malaria

P. falciparum malaria was initially diagnosed using OptiMAL® tests, DiaMed China Ltd. OptiMAL is a reference immunochromatographic rapid diagnostic test (RDT) based on Plasmodium lactate dehydrogenase (pLDH) antigen detection for diagnosis. The anti-pLDH monoclonal antibodies used in the test allow detection of differentiating *P. falciparum* and non-*P. falciparum* infections. The pLDH is a metabolic enzyme secreted by viable parasites only and is rapidly cleared from the

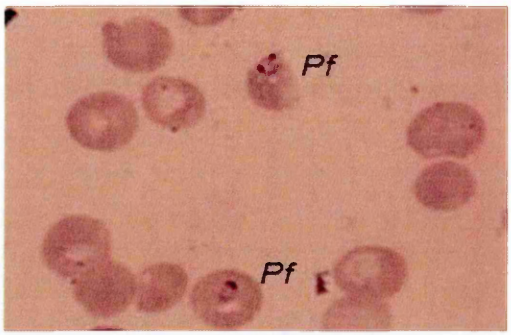
blood stream. Its measurement reflects treatment efficacy. Each test is individually packed and includes all components required for whole blood sampling and test processing. The test can be performed in any field situation after a short period of training. The results are obtained in 20minutes and have both a high specificity and sensitivity performance and good correlation with microscopy.

Figure 2.8. Picture of an RDT based on pLDH metabolic enzyme, which confirms *P. falciparum* infection, and the validity of the test since the control (C) is also positive.



Confirmatory microscopy of Giemsa-stained thick and thin blood films for identification, typing and quantification of malaria parasites [232-234] (Figure 2.9), was performed in children whose initial RDT was positive. Intact parasites were counted against 200 white blood cells (WBCs). However, on slides in which parasite density was high the count was done against 500 RBCs. Parasite densities (parasites per microlitre of whole blood) were calculated against the WBC or RBC count for each patient individually. As a standard procedure, one hundred fields were examined before declaring a slide negative.

Figure 2.9. Blood slide smear showing p. falciparum malaria.



Source: *FEAST trial slide. Peripheral blood film under a microscope remains a gold standard for diagnosis of P. falciparum. Peripheral blood is useful in detection of the early trophozoites, which appear as ring-form and gametocytes. Mature trophozoites are rare in the peripheral blood because they are sequestered in the tissues.*

2.8.2. Complete blood counts (CBC)

Complete blood counts (CBC) were performed on fresh blood samples collected in the EDTA tubes on an automated counter (Beckman Coulter ACT 5diff CP haematology analyser, Beckman Coulter, Inc. Irving Tx 75063, www.beckmancoulter.com). Using these materials and methods we obtained the following scope of laboratory results (Table 2.2).

Table 2.2. Parameters from the Beckman Coulter haematology analyser used in the study:

Cell	Parameter	Reported units
WBC	White blood cell count	x103/μL
RBC	Red blood cell count	x106/μL
Hb	Haemoglobin concentration	g/dL
Plt	Platelet count	x103/μL

2.8.3. ELISA techniques for haptoglobin, methaemoglobin and myoglobin

Haptoglobin, methaemoglobin and myoglobin were detected and quantified from stored samples of plasma and / or urine by sandwich ELISA using commercial kits (Table 2.3). In summary the plates were pre-coated with antibodies against the target proteins; haptoglobin methaemoglobin or myoglobin. The diluted samples

and standards were then added into the antibody-coated wells, incubated and washed to remove unbound proteins. Enzyme-labeled antibodies targeting the specific proteins were then added, after which, plates were incubated and washed several times to remove unbound antibodies. Plates were then developed using a substrate for the enzyme bound antibody [3,3′,5,5′-Tetramethyl benzidine (TMB)], allowing for colour development. The quantity of bound enzyme varied directly with the concentration of the protein in the sample tested, i.e. the absorbance at 450nm is the measure of the concentration of haptoglobin in the test sample.

Table 2.3. ELISA kits, assays, sample and dilutions used

S/N	Assay	Sample type	Dilution	Kit Catalogue number
1	Haptoglobin	Plasma	1:50,000	41-HAPHU-EO1
2	Methaemoglobin	Plasma	1:200	CSB-EO9493h
3	Myoglobin	Plasma	1:10	24-MYOHU-EOL
4	Myoglobin	Urine	1:10	24-MYOHU-EOL

2.8.4. Biochemical assay for determination of plasma and urine haemoglobin

We determined plasma and urine haemoglobin using a laboratory protocol assay based on 3,3′, 5,5′ Tetramethyl Benzidine, acetic acid and hydrogen peroxide. In brief the method operates on the principle that free haemoglobin oxidizes benzidine in the presence of hydrogen peroxide. The procedure involved preparing the sample (either plasma or urine) as described in Table 2.4 below and adding 50µL of diluted samples in duplicate to ELISA plates on which 10µL of TMB were added. The mixture was centrifuged to remove excess TMB and incubated at room temperature for 10minutes. The absorbance was measured at 660nm on spectrometer against a diluent blank. The concentration of haemoglobin

in the samples was then calculated relative to the absorbance of 100mg/dL haemoglobin standard as follows:

$$\frac{(\text{Absorbance of test} - \text{absorbance of blank}) \times (100\text{mg/dL})}{(\text{Absorbance of standard} - \text{absorbance of blank})}$$

Table 2.4. Sample preparation and dilution for plasma and urine haemoglobin assays

S/N	Assay	Normal range	Sample type	Dilution with 10% acetic acid
1	Plasma haemoglobin	<50mg/L	Plasma	6µL plasma: 150µL acetic acid
2	Urine haemoglobin	0.0mg/L	Urine	9µL urine: 150µL acetic acid

2.8.5. DNA extraction

DNA was extracted from frozen samples of whole blood using commercial kits (QIA amp DNA Blood Mini Kits, QIAGEN, www.qiagen.com). The basic principle is that QIAamp DNA Blood Kits are primarily for fast and efficient purification of total DNA and come in two versions depending on volume of blood in the samples. The Midi version is useful for 0.3 - 2mL of whole blood while the Maxi version is useful for 3 - 10mL of blood. The process begins with lysis of the white blood cells and the resultant lysate is transferred onto the QIAamp spin column where the DNA effectively binds to the QIAamp membrane while impurities are effectively washed away in two centrifugation steps. Finally, ready-to-use DNA was then extracted by two stage elution with buffer of pH >9.0 to maximize the

DNA yield. The DNA was then quantified using the Nanodrop ND-8000 spectrophotometer (Thermo Scientific, USA).

2.8.6. Genotyping for red blood cell genetic polymorphisms

2.8.6.1. G-6-PD deficiency

Glucose 6-phosphate dehydrogenase deficiency was genotyped using a novel amplified refractory mutation system (ARMS) PCR method targeting the common African G6PD variants 202 and 376 on the X chromosome using the primers and methodology as follows. The primers for the c.202 G>A polymorphism (rs1050828) were wild type (WT) 202: TGCCCGAAAACACCTTCATCG, mutant type (MT) 202: TGCCCGAAAACACCTTCATCA while the primers for the c.376 A>G polymorphism were (rs1050829) WT376: ACCCCAGGTGGAGGGCATT; MT 376: ACCCCAGGTGGAGGGCATC. In all reactions, we included two additional primers (Lis-F and Lis-R) that target the Lis1 gene [235], as a positive control for the presence of genomic DNA. We used Qiagen Fast PCR master mix with the G6PD (rs1050828 and rs1050829) and Lis1 gene primers added to a final concentration of 0.16μm and 0.2μm respectively. The following PCR conditions were used: denaturation at 95°C for 5 minutes; 30 cycles of 94°C (0:45s), 65°C (1:30s), 72°C (2:00s), and a final elongation step of 72°C for 7 minutes. Because there are three different allelic forms, three separate PCR reactions, one for each of the three allelic forms of G6PD (B, A, and A–), were used each with different primer combinations notably: G6PDB (WT202 and WT376), G6PDA (MT376 and WT202), and G6PDA– (MT202 and MT376). For each of the reactions the presence of the allele of interest was registered when an amplification of a 766bp fragment

in the presence of Lis 1 gene amplicon was read at 2.1kb and absent when only the Lis1 gene amplicon was registered. Our classification of G6PD was guided by phenotypic studies (Shivang Shah, unpublished data) in which G6PD deficient males are those who are hemizygous for the G6PDA– allele while G6PD deficient females are those who are either homozygous for the G6PDA– allele (A–/ A–), or compound heterozygous for the G6PDA– and G6PDA alleles [236].

2.8.6.2. Alpha thalassaemia

We typed for the common African 3.7kb deletional form of □-thalassaemia by PCR, using a modification of the method described by Chong and colleagues [235]. We used QIAgen Fast PCR master mix with each of the following primers added to a final concentration of 0.2μm: 376 (alpha-2 Forward) CCCCTCGCCAAGTCCACCC; 378 (alpha-2 Reverse) AGACCAGGAAGGGCCGGTG; 377 (-3.7 Reverse); AAAGCACTCAGGGTCCAGCG and the Lis1 gene primers as described above and following amplification conditions as described in reference [235]. The amplification products were then run out on a 1% Agarose gel at 95V for 2 hours 30 minutes and reading on an ultraviolet trans illuminator (Gel Doc BioRad, USA). In the presence of normal alpha globin gene, 376 and 378 primer sequences amplify a 1.8Kb product representative of the alpha-2 gene; however, in the presence of the 3.7Kb deletion, 376 and 377 primer sequences produce a 2.1 Kb product. In the heterozygotes both 1.8 and 2.1 Kb products are present. The Lis1 gene is amplified as a positive control for the reaction giving a 2.5Kb product.

2.8.6.3. Sickle cell

We genotyped for the single nucleotide polymorphism on the HBB gene that determines the production of the mutant □s globin associated with HbS using 2 primer sets for detection of the wild type and the mutant alleles as previously described by Waterfall and colleagues [237]. The sickle cell PCR reaction master mix was prepared as follows: deionized water (Sigma), 10X PCR buffer (Invitrogen), 25mM MgCl2 (Invitrogen) 10μM primer [comprising of primers WT-AS, WT-CP, WUT-AS and MUT-CP (Sigma)], 8mM dNTP mix (Sigma), Platinum taq 0.3U/12.5 μL (Invitrogen). This made a single final reaction volume of 10.5μL. At this stage; 1-4 μl aliquots of DNA diluted to 10 ng/μl standard of control or test samples were spotted on the plates and 10.5μl of the master mix was added to each well after which plates were loaded on the cycler (GeneAmp PCR system 9700) programmed as described in reference [237]. Amplification products were run out on a 1% agarose gel at 95V for 2 hours before reading on a UV transilluminator (Gel Doc, BioRad, USA). Presence of a PCR product is based on the whether the 3′ end of the primer sequence is complimentary to the DNA sequence giving products of 517 bp and 267 bp for the wild type (HbA) and the mutant alleles (HbS) respectively.

2.9. Data management

All data from the WSS, SMS, PRODUES and GPS community surveys were cleaned, coded and entered into a database written in Filemaker Pro Version 11.0 (FileMaker, Inc. Santa Clara, California, USA). The data were securely stored in both hard and soft copies. All data were analysed at Mbale RRH Clinical Research Unit (MCRU) using STATA Version 11.0 (STATA Corporation, College Station,

TX). Moreover, the database was designed according to the outline and sections of the CRFs for easy data entry and resolution of data queries.

2.10. Data analysis

I created dichotomous variables for symptoms and signs. For instance among symptoms the following dichotomous variables were created: fever, cough, vomiting, diarrhoea, bloody diarrhoea, convulsions, convulsions lasting more than 30 minutes and passing of dark urine. In addition, among the signs, the dichotomous variables included pyrexia (>37.5^{o}C), hyperpyrexia (axillary temperature of ≥40.0^{o}C), hypothermia (axillary temperature of ≤36.0^{o}C), pallor, clinical jaundice, hypoxia (oxygen saturation <90%), respiratory distress (increased work of breathing), impaired consciousness (any of VPU on the AVPU scale), prostration, coma, severe tachycardia (age graded), temperature gradient and capillary refill time [CRT (>2sec)]. Among the laboratory characteristics, dichotomous variables created included malaria slide positive, severe anaemia (haemoglobin <5g/dL), hypoglycaemia (glucose <2.5mmols/L); acute renal impairment (blood urea nitrogen (BUN) >20mmols/L); metabolic acidosis (lactate >5mmol/dL) or base excess of -8 or higher); hyponatraemia (sodium <130mmols/L); hypernatraemia (>145mmol/L); hypokalaemia (<3.5mmols/L); hyperkalaemia (>4.9mmols/L) and elevated anion gap (>11.0mEq/L). Malaria meant a positive blood slide in the WSS while both positive slide and positive OptiMAL® rapid malaria diagnostic tests were considered together to make malaria diagnosis in REDUES, SMS and PRODUES studies. Continuous variables were compared using Student's t-tests and by ANOVA while categorical variables were analysed using x^2 tests. Variables associated with statistical *P-values* of <0.05

at univariate were considered statistically significant and were included in the multivariate analysis. The results were presented in tables, graphs and diagrams. These dichotomous variables were analysed and their proportions among children who survived and those who died were computed. I compared proportions of these dichotomous variables between survivors and deaths and computed their odd's ratios and *P-values* for mortality. Any *P-value* <0.05 was taken as statistically significant. Continuous variables were summarized using medians with interquartile range (IQR) or means with 95% confidence intervals (CI) as appropriate. Geometric means were calculated for malaria parasite density. These continuous variables were compared using Student's *t*-tests and by ANOVA. The diagnoses were summarized, their proportions calculated and presented in tables or figures.

2.11. Case management

At the PACU all patient management was done using two protocols: the ETAT (emergency triage and treatment) guidelines [238], were followed in triaging all sick children on arrival into those with emergency signs, with priority signs, or non-urgent cases. In addition ETAT guidelines were used to steer immediate emergency and life support treatment as the first step and means of stabilizing patients. Once the emergency and priority cases were stable, underlying causes for their admission were definitively treated using the Uganda National Treatment Guidelines 2010. These included but not exclusive to: Antibiotic treatment for bacterial diseases such as: pneumonia, meningitis, infective diarrhoea, sepsis, dysentery, and urinary tract infections. During the period of this study, antimalarial treatment centered on I/V Quinine especially for severe malaria in

which, quinine was given intravenously at a dose of 10mg/Kg body weight in 10mL of 5% dextrose/Kg body weight as a slow infusion over 4 hours period and repeated every 8 hours until the patient could tolerate oral medication in the same dose for a total of 7 days. Malaria presenting as co-infection without features of severity was treated with oral artemesinin-based combination antimalarial, Artemether/Lumefantrine widely marketed as (Coartem). Blood transfusion for severely anaemic patients (Hb <5.0g/dL) dosed at 20mL of whole blood per Kg body weight was given to all deserving patients. In addition, oxygen therapy for hypoxic patients (O_2 saturation <90%) was given. Furthermore, hypoglycaemic patient (blood glucose <2.2mmol/L) were given 2mL of 25% dextrose per Kg body weight. Dehydration was treated with fluids according to the WHO protocols for some dehydration (Plan B) and severe dehydration (Plan C). Linkages and referral to other units for specific care were done including referral to the Paediatric Infectious Diseases Clinic (PIDC) for HIV infected children, TB clinic for children for children suspected with TB, Nutrition Unit for malnourished children and physiotherapy department for children requiring rehabilitation services. Supportive treatment including temperature control in those with temperatures >38.5^{o}C was done with paracetamol as antipyretic drug with intermittent tepid sponging. Standard of care nursing available was offered.

Management of patients with dark urine syndrome has been problematic throughout the history of this illness in Eastern Uganda. There are no local or national treatment guidelines for slide negative dark urine syndrome, which seems to be predominant among our patients. The approach of treatment of "the underlying cause" in patients with dark urine syndrome was widely adopted but

the exact cause of this cryptogenic illness remains unknown to date. Many patients with dark urine syndrome were treated with antimalarials because of the available literature linking the condition to *Plasmodium falciparum* malaria with slide positive, low parasitaemia or no parastaemia [179, 180, 239, 240]. In addition, steroids were used in some cases despite lack of evidence for their indication, safety and efficacy this condition.

2.12. Follow- up

Other than the REDUES and PRODUES, all other studies were not primarily designed to have follow up component for survivors. However, follow up of cases of severe malaria with neurological sequelae, asthma, sickle cell anaemia, congenital heart condition and others who required additional medical review appointments and rehabilitation at discharge were followed up at the regular paediatric clinic as part of ongoing patient care activities at the department of paediatrics.

2.13. Ethical considerations

2.13.1. Ethical clearance

Prior to the launch of my research projects, I obtained ethical approvals to conduct the surveys and storage of samples for further analyses abroad from the Mbale Regional Hospital Institutional Review Committee [MRHIRC (REIRC. 005/2010), (see appendix)] and Uganda National Council for Science and Technology [UNCST (HS. 970), (appendix)].

2.13.2. Consent form

Because severe malaria and dark urine syndromes are paediatric medical emergencies, I adopted a consenting process as described by Maitland *et al* and Molyneux *et al* in the FEAST Trial [241, 242]. Basically, this involved a two-stage consent process in which initially an assent was obtained from the parent or guardian to allow the child to be included in the study without delaying treatment. When the patient was stable, full informed consent was given and either the parent or a guardian signed the informed consent form.

2.14. Summary of the pre-study procedures

To achieve the above, I put systems and processes in place to enable me use the materials, perform the methods and techniques as stated in my study manual of operations and standard operating procedures.

I did a pilot study January - April 2011. The purpose of which was to test the data collection tools including the CRFs, informed consent forms and HCC. In addition, during the month of February 2011, I worked with a team from KEMRI-WTRP and collected 27 samples from patients with dark urine syndrome and retrieved another 27 samples from the FEAST trial and analysed them. These were done to establish the feasibility of doing the planned laboratory tests and procedures in either Mbale and/or Kilifi (KWTRP). Moreover, the costs of each test were established and were used as a guide to tailor my PhD research budget to a manageable level. Conversely, I did study site preparations including but not restricted to renovation of the PACU, procurement of point of care equipment, putting in place patient flow systems which included: reception, triage, emergency

and consent areas. I procured and installed lockable cabinets for safe storage of equipment and study documents. Also, I procured data collection tools and availed them throughout the study period. In addition, I developed and made simple easy to use manual of operations (MOP) and standard operations procedures (SOP) that we used as a study team throughout the study period. Furthermore, training of the study team on ETAT model of care, basic research methods including data collection, good clinical practice and research ethics were done. I also put in place data analysis plans for my studies ensuring that these plans were in line with the study objectives, hypotheses and the study questions. To ensure reliable sample storage, I made a memorandum of understanding with the Joint Clinical Research Centre (JCRC) for storage of my study samples and subsequently made a well-negotiated material transfer agreement (MTAs) with KWTRP. Lastly, I kept the meetings, training and plan memoires throughout the study period. I used these memoires to track my study progress as well as important action points with the supervisors.

CHAPTER 3: A description of paediatric admissions at Mbale Regional Referral Hospital

3.0. Abstract

Estimating the burden of disease in the Sub Saharan Africa (SSA) remains a problem especially in countries relying on their health system's routinely collected data. I designed a prospective paediatric admissions ward surveillance study (WSS) of illnesses among children admitted to the paediatric acute care unit (PACU) of Mbale Regional Referral Hospital (Mbale RRH) to understand the spectrum, burden of paediatric illness including malaria, DUS and their outcomes.

Between May 2011 and April 2012, we conducted a clinical survey on 10,208/23,217 (44.0%) of the admissions among children aged 0 - 144 months. Heavy case burden was among children below 5 years, 87.2% (n=9,510). The male: female ratio was 1.25: 1; the difference may have been due to community health seeking behaviours. The commonest symptoms included: fever 8,784/10,208 (86.1%), though non-specific may point at infectious causes, cough 7,004/10,208 (68.6%) was common and included children with malaria suggesting either co-infection with bacterial and/or viral causes or the rarely reported malaria associated cough. Gastrointestinal symptoms were common too, vomiting 5,173/10,208 (50.7%) and diarrhea 3,395/10,208 (33.3%) featured frequently but the expected associated signs of dehydration including sunken eyes were not equally common suggesting self limiting underlying causes such as viral aetiology. It was surprising that convulsions 1,132/10,208 (11.1%) were common, though this study was not able to differentiate febrile from non-febrile

convulsions. There was an acute history of dark urine in 1,087/10,208 (10.6%); of these children 666/1,087 (61.3%) had a previous history of dark urine suggesting recurrent phenomenon in the same individuals. Though most patients 8,784/10,208 (86.1%) complained of fever, measured temperature was in 5,985 (59%) of whom pyrexia (>37.5^{o}C) was in 3,669/5,985 (61.3%). Pallor a sign of anaemia was common 3,290/10,208 (32.2%), respiratory distress accounted for 1,968/10,208 (19.3%), while severe tachycardia (age graded) was in 1,762/10,208 (17.3%) and capillary refill time >2sec; 1,499/10,208 (14.7%).

In total 6,714/10,208 (65.8%) had *Plasmodium falciparum* malaria of which 662/6,714 (9.9%) were in children with features consistent with severe and life-threatening malaria. All severities of anaemia (Hb <10g/dL) were common 2,020/10,208 (19.8%) of which severe anaemia (Hb <5.0g/dL) accounted for 1,655/2,020 (81.9%) or (16.2%) of all admissions. HIV rate was low 201/10,208 (2.0%) compared to the background prevalence of 5.3% in the region. Total in-hospital mortality during the period of the survey was high 655/10,208 (6.4%). Data were further analysed for 9,551/10,208 (93.6%) patients with complete data on age, of whom neonates (<1months old) comprised of 941/9,551 (9.9%) and presented commonly with fever 847/941 (90.0%), cough 619/941 (65.8%), vomiting 493/941 (52.4%) and diarrhoea 322/941 (34.2%). I elicited common signs among the neonates, including: pyrexia (>37.5^{o}C) 202/355 (56.9%), pallor 478/941 (50.8%), clinical jaundice 259/941 (27.5%), elevated capillary refill time (>2 seconds) 216/941 (22.9%) and respiratory distress 144/941 (15.3%). Whereas, the symptoms were general and potentially would be from a wide range of underlying causes, at admission, the common diagnoses were few and included

neonatal sepsis 693/941 (73.6%), diarrhoea 322/941 (34.2%) and pneumonia 228/941 (24.2%). It was surprising to note that neonatal sepsis was the commonest diagnosis though common symptoms including diarrhoea and vomiting are typically not associated with this diagnosis. These high rates of gastrointestinal symptoms may be due to community infant feeding practices including early introduction of supplementary feeding within the first month of life. The case fatality rate among neonates was high 124/941 (13.2%).

Conversely, children aged >1 month were a majority and comprised 8,610/9,551 (90.1%). Their common symptoms at presentation included fever 7,592/8,610 (88.2%), cough 6,142/8,610 (71.3%), vomiting 4,556/8,610 (52.9), diarrhoea 3,002/8,610 (34.9%) and history of the previous transfusion in 1,444/8,610 (16.8%). The common signs were pyrexia 3,063/5,630 (54.4%), pallor 2,682/8,610 (31.1%) and respiratory distress 1,698/8,610 (19.7%). Among the diagnoses, the common ones included malaria 5,883/8,610 (68.3%), diarrhoea 3,002/8,610 (34.8%) and pneumonia 2,617/8,610 (30.4%). The case fatality rate (CFR) in children >1 was lower 321/8,610 (3.7%) compared to that in neonates.

In conclusion, malaria, pneumonia and diarrhoea were common causes of admission among children older than one month of age. Overall mortality was high (6.4%) but higher case fatality rate was in neonates 13.2% v 3.7% in children >1 months old.

3.1. Introduction

The Sub Saharan Africa (SSA) remains heavily burdened with infectious diseases compared to the rest of the world [243]. Accurate estimates of the burden of

disease in the SSA remain lacking [243]. In the region, most national health statistics are based on routinely collected data, which often have their limitations [244, 245].

In most health units, especially in resource-limited settings, use of integrated management of childhood illnesses (IMCI) for care of the under 5 year olds is common [246, 247]. The IMCI guidelines were initially meant to empower lower cadre health workers in the peripheral health units to appropriately identify children at high risk of mortality, initiate treatment and refer them for appropriate definitive case management in referral centres. However, these guidelines have now diffused into the district and referral hospital settings where up to 10% of the doctors have been trained on IMCI skills and practices [248]. Moreover, IMCI uses nonspecific disease classifications such as "very severe disease" meaning any childhood severe illness qualifies this classification, for example; severe malaria, pyogenic meningitis, severe acute malnutrition, severe dehydration and/or severe pneumonia [249-254]. Consequently, because of its syndromic approach, the classification of diagnoses and mortality for common paediatric illnesses are neither distinct nor comply with the ICD-10 code; thereby limiting comparison of data across different settings. Despite these shortcomings, this syndromic approach has been widely accredited for improving child health in resources limited settings [249-253].

At community level there are few data that have described disease burden [255, 256]. The rolling out of the IMCI model to community level remains challenging

[248]. Against this background, estimating the burden of disease in the SSA at community level is complex, costly and culturally difficult [257-260].

3.2. Review of common causes of admissions in childhood

Infectious diseases remain the leading causes of morbidity and mortality in the SSA. In Liberia, Couto *et al* did a retrospective study from March 2009 to October 2010 and found that of the 8,254 patients admitted to a paediatric referral facility, the overall mortality was 531 (6.4%) of whom 90% occurred in children <5 years old [261]. They also noted that a majority of deaths occurred within 24 hours of admission, similar to reports by earlier researchers [8, 24, 113], and more recently in the FEAST trial [159]. Furthermore, Couto *et al* noted that a majority of the deaths (76%) in their study were due to infectious diseases [261]. Also, Huerga *et al* conducted a retrospective study from January - July 2005 at Monrovia Referral Hospital in Liberia. They found that; of the 1,509 paediatric admissions, the fatality rate was high, 197 (13.1%) with paediatric infections accounting for 66% of the deaths. The case fatality rates were higher; 18% in neonates who mainly had neonatal sepsis 47.0%, respiratory distress 24.0% and prematurity 18.0%. On the other hand, among children aged >1month, the main causes of deaths were acute respiratory tract infections 27.0%, malaria 23.0% and severe acute malnutrition (SAM) 16% [262]. These finding may have been influenced by prolonged civil conflict in Liberia, a situation that may have affected the country's health services including public health interventions such as immunization. In the Democratic Republic of Congo (DRC), Greenberg *et al* in a retrospective hospital based surveillance of paediatric admissions during the period June 1985 to May 1986, found that of the 6,208 admissions 2,374 (38.2%) had malaria; with malaria case

fatality rate (CFR) of 500 (21.1%) [263]. These data have limitations since they were retrospectively collected. Even then, this study indicated a high CFR especially in children <5 years of age and was thought be associated with malaria, as is often the case in high transmission areas [1].

There are few data on comprehensive prospective paediatric admissions surveillance. Where they are available, their quality is poor, for example, English *et al* in a prospective study assessed inpatient paediatric care in the 14 first referral level hospitals in 13 districts in Kenya. They noted that routine data had missing values, some diagnoses were avoided including HIV/AIDS and lacked standardised definitions [245]. Moreover, the authors found that there was considerable variations with routine statistics in which mortality ranged from 4% to 15%, case fatality rates due to anaemia ranged from 3% to 46%, malaria severity signs were not consistently documented and varied per hospital for instance impaired consciousness ranged from 0 - 100%, while respiratory distress ranged from 9 - 77% [245]. However, in some selected health establishments and research centres in the SSA, estimates of morbidity and mortality have been based on specific disease conditions, rather than a whole admission cohort. For instance, there is some data available on; malaria [264-268], diarrhoea [265, 269], malnutrition [270-272], HIV/AIDS [273-277], tuberculosis [278-280] and meningitis [281]. Complimentary to single disease reports are the health management information systems (HMIS), widely implemented to strengthen the district health systems through informed decision making while providing data to help understand the disease burden in resources limited countries [282]. In Malawi, outside of the perinatal period, the burden of disease occurring in children 2 - 5

years is similar to that in the <1 year of age and includes: malaria, anaemia, pneumonia, measles, diarrheal diseases, malnutrition and other causes [283]. Though no proportions were tagged to each condition, it is likely that the burden of disease in the <1 year olds is higher owing to high risk in this age group due to poor immunity and increased background burden of infectious diseases.

In many settings, data are scanty on facility-based disease surveillance. Iriso *et al* in a retrospective study on discharge records for the period 1992 - 1998 in Lacor Hospital in Northern Uganda, reported that of the 40,564 paediatric patients discharged, the various conditions accounted for different proportions including malaria 41.2%, malnutrition 10.1%, pneumonia 8.3%, measles 7.1%, gastro-enteritis 4.2%, septicaemia 4.2%, meningitis 2.5%, URTI 2.1% and anaemia 1.9%; in that order of importance [284]. These findings were limited by the retrospective design of the study and only reported data from survivors hence may not fully represent the actual burden of disease with high in-hospital mortality. However, they represent initial data for identifying common causes of primary admissions to this and other referral facilities in the country. Similarly, Sievers *et al* in Rwanda conducted a retrospective study on all admissions between two consecutive malaria peak seasons of December 2005 - February 2006 and December 2006 - February 2007. In August 2006 the Rwanda government introduced community malaria intervention involving mass distribution of long lasting insecticide treated bed nets and community distribution of antimalarials in the catchment community of Rwinkwavu district hospital in the rural Eastern Province of the country. They analysed 551 paediatric admission records during this period and found pre v post - intervention admission rates to be 322 (58.4%) v 229 (41.6%),

the total suspected malaria admissions pre v post - intervention were 287 (89.1%) v 150 (65.5%) and the total other admissions pre v post - intervention was 35 (10.9%) v 79 (34.5%), respectively [285]. These results compare favourable with findings by Ceesay *et al* in The Gambia for the period 1999 - 2007, when there were mass interventions for the control of malaria in the country. Though Ceesay *et al* did not report on the trends on all other admissions, they noted that the mean haemoglobin concentration among admissions increased by 1.2g/dL in the period 2004 - 2007 when malaria prevalence decreased. In addition they reported that the mean age in years (95% CI) of paediatric malaria admissions increased from 3.9 (3.7–4.0) to 5.6 (5.0–6.2) [286], suggesting that malaria; both symptomatic and asymptomatic in malaria high transmission areas may be the main determinant of pre-admission haemoglobin level in children. Moreover, findings by both Sievers *et al* and Ceesay *et al* were consistent with those in a more recent study by Meara *at al* in the coastal Kenya [287].

In Uganda, the Millennium Development Goals (MDGs) for child health aimed at reducing childhood mortality to 56/1000 live births by 2015 is unlikely to be met, since estimates of the under-five (U5) mortality remain high; 117/1000 live births [288]. The country's majority of the post-neonatal deaths have been attributed to malaria, respiratory tract infections, malnutrition, diarrhoeal diseases and HIV [288]. With only limited time left to the end of the initial MDG targets' deadline, a likelihood of setting up new targets remains a possibility. Nuwaha and Mukulu in their analysis and projections for attainment of <5 mortality in Uganda concluded that the current annual average reduction rates (AARRs) for the <5 mortality rates of -1.05 - 3.05% were below the 4.4% required to attain MDG4 by 2015 [289].

Similar thoughtful analyses are important in setting new targets, and for tracking MDGs' progress.

Table 3.1. Summary of Neonatal, Postnatal and Childhood mortality in Uganda

3.1.a Under 5 Mortality rates for 5 decades from 1970 - 2010

Year	1970	1980	1990	2000	2010
U5MR	190.4	185.9	167.8	141.1	116.7
	(183.8 – 196.3)	(181.5 – 190.7)	(163.5 – 172.1)	(136.8 – 145.6)	(105.0 – 127.5)

3.1.b Neonatal, Postnatal and Childhood mortality for 1990 and 2010

Year	Neonatal	Postnatal	Childhood
1990	42.2	56.1	79.5
	(31.2 – 55.3)	(43.8 – 68.0)	(61.7 – 96.3)
2010	31.9	40.5	49.0
	(23.2 – 42.0)	(31.5 – 49.9)	(34.1 – 63.0)

Legend: *U5MR = Under 5 Mortality Rate (confidence interval)/1000 by decade.*

Neonatal, postnatal and childhood mortality (confidence interval)/1000 in 1990/2010

Source: *Neonatal, postnatal, childhood, and under-5 mortality for 187 countries, 1970–2010: a systematic analysis of progress towards Millennium Development Goal 4 [288]*

In Uganda over 90% of the population lives in areas highly endemic for malaria. The Uganda Ministry of Health (MOH) estimated in 2005 that malaria accounted for 25 - 40% of outpatient attendances, 20% of all admissions and 9-14% of inpatient deaths occurred that year, 90% of these in children under 5 years [290]. Whilst some areas of Africa are witnessing a decline in malaria [291], these have largely been areas in which the background transmission intensity is relatively low and thus rapid scaling up of preventative measures and early treatment interventions have resulted in impressive declines in malaria disease burden. Since 2005 when baseline transmission was high together with inadequate scaling up of interventions, it has been estimated that 70,000 - 110,000 malaria deaths occur in Uganda, annually [290]. These statistics are considered as underestimates since many cases and deaths remain unreported and the existing reporting system itself is largely lacking. In contrast to reports indicating a declining incidence of malaria transmission in some parts of Africa [291], Okiro *et al* recently reported that periodical data assembled for January 1999 to December 2009 on paediatric admissions in five Ugandan hospitals and their catchment areas indicated a crescendo in malaria admission rates. In addition, they noted considerable monthly increments in the average malaria admission rates at four out of the five hospitals ($P<0.001$). Furthermore, during the study period, Okiro *et al* corroborated that despite increase in insecticide-treated net coverage from <1% in the year 2000 to 33% by 2009 at all hospitals studied, malaria related admissions had significantly increased from 47% in the year 1999 to 350% by 2009; with only 40% corresponding increase in access to nationally recommended therapies [52]. These data suggests that the incidence of malaria in Uganda is actually increasing

[292]. Idro *et al* in their study on severe malaria in children in areas with low, moderate and high transmission intensity in Uganda recruited 617 children with severe malaria presenting to three hospitals in areas with different transmission intensities as follows: the hospital with very low transmission accounted for 51 (8.3%) patients, the one with moderate transmission had 367 (59.5%) patients and that in very high transmission with recent EIR of >100 infective bites per person per year [229]; had 199 (32.2%) patients [22]. These data have some limitation since they neither account for the overall numbers of patients admitted in each setting nor comment on the willingness of the local teams to collect data. Nonetheless, these data point to the fact that, paediatric malaria remains an important public health problem across Uganda. Few data are published on the burden of malaria in children in Ugandan hospitals. Further understanding of these is important for immediate case management and in the longer term to understand the effects on the disease epidemiology once effective control and treatment interventions are implemented.

Pneumonia is a common condition in children and a common cause of hospital admissions and in hospital mortality [293]. In Uganda, few data on pneumonia have been published, even fewer data exist after the introduction of Haemophilus influenza B (HiB) vaccine in 2002 and pneumococcal conjugate vaccine (PCV) in the period 2012-2014. In developed countries, as reported by Farha *et al*, the burden of community-acquired pneumonia is low, in the range 10-15/1000 children annually with a corresponding lower hospital admission rate of 1-4/1000 annually; of these, a majority are children under the age of 5 years [294]. The aetiology is often difficult to establish but up to now bacterial causes especially

Streptococcus pneumoniae remains an important cause [294]. In-hospital morbidity and mortality attributable to pneumonia have shown a fall after the introduction of an effective vaccine in developed and some developing countries [294, 295]. On the other hand viral aetiology especially influenza A, B, respiratory syncytial virus (RSV) and Parainfluenza 1, 2 and 3 are the most common viruses associated with childhood pneumonia [294, 296]. In the developing countries incidence and prevalence of pneumonia has been influenced by HIV epidemic especially for the period 1998 - 2005. Prior to the advent of antiretroviral therapy (ART) and Cotrimoxazole prophylaxis, the clinical spectrum of pneumonia in children was also influenced by HIV sero status. For example, Bakeera-Kitaka *et al* in their prospective study on the prevalence of Pneumocystis carinii pneumonia (PCP) among 121 patients aged 2 - 60 months with severe pneumonia at Mulago Hospital in Kampala, found that the prevalence of PCP was 20 (16.5%) of whom 12 (60%) were infants < 6 months of age. The overall prevalence of HIV in this study was 43 (35.5%) of whom 18 (42%) had PCP and only 2/78 (2.6%) of the HIV negative had PCP. Mortality in the PCP group was higher 40% compared to 20% in those without [297].

Nantanda *et al* conducted a four months (December 2005 - March 2006) prospective study on aetiology of severe pneumonia among 157 children aged 2 - 59 months at the acute care unit at Mulago Hospital, Kampala Uganda. In their isolates, they found that blood cultures had a low (15.9%) yield for bacteraemia but *Staphylococcus aureus* (36%), which possibly was a contaminant, and *Streptococcus pneumoniae* (28%) were the most common bacteria isolated. However, sputum cultures had a higher (50%) yield, with the common organisms isolated

being *Streptococcus pneumoniae* (45.9%), *Haemophilus influenza* (23.5%) and *Klebsiella species* (22.4%) [298]. Furthermore, they reported overall case fatality of 15.5%. In their study, Nantanda *et al* identified poor prognostic indicators including signs of very severe pneumonia OR (95% CI) 12.9 (2.5-65.8), hypoxaemia (SaO_2 <92%), OR (95% CI) 4.9 (1.2-19.5) and severe acute malnutrition OR (95% CI) 16.5 (4.2-65.5) [298]. In yet another study, Kallander *et al* conducted a community-based surveillance in Iganga district in Eastern Uganda where verbal and social autopsies were done on 167 deaths among children aged 1 - 59 months from November 2005 to August 2007. They reported that 27% (n=167) died of pneumonia of whom 50% died in the hospital and 30% died at home. Furthermore, they reported that the median duration of pneumonia related illness was 7 days; noting inappropriate treatment among 52% of the children who received antimalarials as first treatment, while only 27% had antibiotics initially [299].

The roll out of *Streptococcal* conjugate vaccine across Uganda has shown positive public health achievements. Kisakye *et al* in their review of acute bacterial meningitis surveillance data for children aged <5 years from 3 sentinel surveillance sites in the three Ugandan districts of Gulu, Iganga and Kampala for the period 2001 to 2006 reported that a total of 14,388 probable acute bacterial meningitis cases were observed, of which 331 (2.3%) were confirmed isolates. The most common cause identified was *S. pneumoniae* 35% (n=331) with case fatality rate of 19%. They computed the yearly pneumococcal meningitis incidence for two districts and found: 3 - 20/100,000 population in Kampala versus 28 - 42/100,000 population in Gulu respectively. The most frequent serotypes were 6A/6B (40%)

of which 43% of isolates were serotypes in the available 7-valent pneumococcal conjugate vaccine and 70% were in the 13-valent-pneumococcal vaccine [300]. The unusual finding in this study was the substantial (83.0%) intermediate resistance of *S. pneumoniae* to penicillin [300], yet this is the first line treatment recommended for *S. pneumoniae* in Uganda (Uganda National Treatment Guidelines, 2010). In another study involving 17 districts in Uganda, Lewis *et al* reviewed data to determine *Haemophilus influenzae* (HiB) meningitis among children aged 0 - 59 months for the period July 2001 to June 2007. They found that of the 13,978 children with suspected bacterial meningitis, 269 (%) had confirmed HiB meningitis. They demonstrated a great decline from 69 patients in the pre-vaccine year (2001-2002) to three in 2006-2007 with a corresponding drastic drop in incidence in HiB from 88/100,000 in the year before vaccine introduction to zero by the 5th year of the vaccination programme [301]. Furthermore, they reported that vaccine effectiveness for 2 or more doses was 93% (95% CI 69-99) against confirmed HiB meningitis and 53% (95% CI: 11-68) against purulent meningitis due to other causes [301].

Diarrhoea has widely been known to be a common cause of poor health in children in resources limited settings. Tumwine *et al* surveyed 1,015 households in 33 sites in Uganda, Tanzania and Kenya for the period 1967 to 1997 and found that the prevalence of diarrhoea, one week before the survey had increased from 6% to 18% in Kenya and from 16% to 21% in Uganda. However, in the same period these researchers noted a declined from 11 to 8% in Tanzania. The factors associated with diarrhoea included poor hygiene (defined as unsafe disposal of faeces and wastewater), low education level of household head, open surface water sources

or wells and high cost of water used for cleaning in the households [302]. In Kenya, Tornheim and Feikin in their study on epidemiology of hospitalization with diarrhoea in rural Kenya found that diarrhea accounted for 11.2% (n=2,158) of admissions with an annual incidence among <5 year olds at 550/100,000 and that among >5 year olds at 216/100,000 respectively. Furthermore, they reported that the incidence was highest in infants 1,138/100,000 but decreased in older children [303]. In the coastal part of Kenya, in the Kilifi District Hospital, Nokes *at al* conducted a prospective surveillance for group A rotavirus between 2002 - 2004 with a nested unmatched case-control study in which cases were children <13 years with diarrhoea (n=3,296) and control were children <13 years without diarrhoea (n=620). They aimed at describing incidence, complication and outcomes of rotavirus type A. The authors found that the overall prevalence of diarrhoea in this 3 year prospective study was 3,296/15,347 (22%) found that of the 2,039 cases of diarrhoea tested for rotavirus type A, 588 (29%) tested positive. Infants constituted a great proportion 372/588 (63%) [304].

In Mulago hospital, Kampala, Bitarakwate *et al* in a prospective comparative study aimed at describing factors associated with persistent diarrhoea in children aged 6 - 36 months, found that when compared to children without diarrhoea the prevalence of zinc deficiency in patients with persistent diarrhoea was 66.7% vs. 47.9% and the mean serum zinc level in the children with persistent diarrhoea was low 5.83mol/L v 8.99mol/L, (P<0.001). Furthermore, they found that hypoproteinaemia was significantly associated with low serum zinc levels (P=0.03) [305]. At community level, Nasinyama *et al* in their study of household risk factors for acute diarrhoea among residents in Kampala, an affluent

community than many other parts of Uganda, found significant associations between acute diarrhoea with those who drank raw chicken eggs (OR=99; $P<0.01$), household infestation with pests (OR=2.6) and poor household income [306]. Conversely, Mbonye in a similar study but in a rural and less affluent community investigated risk factors for diarrhoea among 300 mothers with children aged less than two years. He found that the prevalence of diarrhoea among children was 40.3% and the risk factors for diarrhoea in children included unimmunized child (OR=2.8; $P<0.001$), lack of a pit latrine in the household (OR=1.4, $P<0.03$), inadequate knowledge of reconstituting oral rehydration salts (OR=1.7; $P<0.01$), garbage littered compounds (OR=2.6; $P<0.001$), poor hand washing practices: upon visiting latrine (OR 1.8; $P<0.03$), and before touching or preparing children's food (OR=1.4, $P<0.04$)[307]. The causative organisms for acute diarrhoea vary widely. Among the viral causes rotavirus is the commonest as reported by Nokes *et al* in the coastal part of Kenya [304]. Other viral causes include enteric adenoviruses and enteroviruses [308]. Bacterial causes include a broad range of organisms in the class enterobacteria including *salmonella, enteropathogenic E. coli* (EPEC), *Campylobacter jejuni, enterotoxigenic E. coli* (ETEC) and *Shigella* [308, 309]. Chronic diarrhoea on the other hand is mainly caused by parasites including: *Giardia, Cryptosporidium* and *Cyclospora* [310].

Malnutrition is a common cause of morbidity and mortality especially in the developing, resources limited countries. Kikafunda *et al* in their study on risk factors for early childhood malnutrition in Uganda found that 21.5% of children surveyed were sick and specifically 3.8% had kwashiorkor and 5.7% had marasmus [311]. In addition, they reported that a high (23.8%) proportion of

children were stunted, another 24.1% were underweight and 21.6% had a low MUAC, a majority of them were of poor economic status [311]. Owor *et al* conducted a matched case - control study [cases n=66 and controls n=66] in Mulago hospital in Kampala to determine the socio-economic risk factors for protein energy malnutrition (PEM) in children under 5 years of age. They found that severe PEM was correlated with age of the caretaker (P=0.005), poor housing OR (95% CI) 2.44 (1.13 - 5.32), deprived breast feeding OR (95% CI) 3.22, (1.31-8.02), incomplete immunisation in a child OR (95% CI) 3.68 (1.53 - 9.011), no household land ownership OR (95% CI) 4.62 (2.09 - 10.3), and no household ownership of livestock OR (95% CI) 13.65 (3.60 - 60.84). There was no association between severe PEM and the level of formal education of the caretaker [312].

The rarely reported childhood conditions in Uganda have a regional tendency including infections such as *schistosomiasis* in the shores of lake victoria, *leishmaniasis* in the Karamoja region in the North-Eastern part, *trypanosomiasis* in the Busoga region in the Central-Eastern region and *onchoschiais* in Northern part of the country. Epidemiological factors such as older males play a role possibly due to occupational exposure such as fishing, hunting, irrigation and herding animals, which are predominantly male dominated activities in Uganda. Congenital abnormalities other than congenital cardiac conditions are rarely reported. Furthermore, non-communicable burden of disease have been reported but they remain an infrequent cause of hospital admissions in children.

The neonates (0 - 28 days of age) are at higher risk of most illnesses, clinical complications and poor outcomes. Ondoa-Onama *et al* conducted a prospective

descriptive study on immediate outcomes in newborn babies with low APGAR (Activity, Pulse, Grimace, Appearance & Respiration) scores in Mulago Hospital, Kampala Uganda, from September to October 1999. They compared children with low APGAR scores (defined as ≤6) and babies with normal APGAR score (7 - 10); at one and five minutes after birth. They found that the prevalence of low APGAR score at one minute was 8.4%, and as expected was higher than at five minutes 2.8% [313]. Overall poor outcomes were registered in 57.3% children with low APGAR scores among which were a mortality of 12.1% and clinical complications in 45.2%. The spectrum and prevalence of clinical complications varied, ranging from 21.8% for hypoxic ischaemic encephalopathy (HIE), 12.9% for hypoxaemia, 16.9% for hypoglycaemia to 4.8% for aspiration pneumonia [313]. Dualistic maternal and newborn's sets of factors were importantly associated with low Apgar scores. Maternal factors included primiparity OR [95% CI (*P*)] 1.99 [1.16 - 3.43 (*P*=0.008)], abnormal delivery OR [95% CI (*P*)] 0.61 [0.33 - 1.10 (*P*=0.0330], age OR [95% CI (*P*)] 0.94 [0.89 - 1.00 (*P*=0.034)], and ill health during pregnancy OR [95% CI (*P*)] 1.86 [0.98 - 3.56 (*P*=0.05)]. Conversely, birth injuries OR [95% CI (P)] 4.23 [1.41 - 12.6 (*P*=0.012)] and cord accidents OR [95% CI (*P*)] 4.31 [1.02 - 18.2 (*P*=0.049)] were the newborn factors. On outcomes they found birth injury, hypothermia, hypoglycaemia, hypotension, aspiration pneumonia, hypoxaemia and severe birth asphyxia were poor prognosticators [313]. Risk factors for each of the poor outcomes identified by Ondoa-Onama [313]; have rarely been studied elsewhere in Uganda yet they may have population specific significance. However, Byaruhanga *et al* in a cross sectional descriptive study on the prevalence and risk factors for neonatal hypothermia at St. Francis Hospital Nsambya, in a

peri-urban area of Kampala, reported that of the 300 newborn babies whom they measured parallel tympanic and rectal temperature at 10, 30, 60, and 90 min postpartum; the prevalence of hypothermia (defined as temperature ≤36.5^{O}C) notably at 10 min was (29%), 30 min was (82%), 60 min was (83%), and 90 min was (79%) respectively [314]. Contact with the mother was preventive to hypothermia. The proportion of newborn babies with hypothermia and without contact with their mothers was higher compared to the non-hypothermic babies 87.0% vs. 75.0%; (P=0.03). In their study, whereas the mean birth weight of 3,218 g was within the normal rage for African new born babies, they found low birth weight was a risk factor for hypothermia [9/86 (10.0%) v 9/209 (4.0%); P=0.08] [314]. Like Ondoa-Onama *et al*, Byaruhanga *et al* found young maternal age was associated with hypothermic newborns than in those without hypothermia (P=0.025). However, they found that parity, preterm deliveries, time of delivery, early rapture of membranes were not significantly different between hypothermic and non-hypothermic newborns [314]. Preterm babies are at higher risk of a number of complications. Namiiro *et al* when investigating weight recovery among discharged preterm and low birth weight (LBW) babies in the Kangaroo Method Clinic in Mulago Hospital, Kampala; conducted a descriptive cross sectional study from January to April 2010. They found that of the 235 LBW infants, 113 (48.1%) had not regained their birth weight by day 21. The independent factors associated with failure to gain weight by expected day 21 included hospitalization for more than 7 days AOR (95% CI) 4.2 (2.3 - 7.6); P<0.001 and delay initiating the first feed beyond 48 hours AOR (95% CI) 1.9 (1.1 - 3.4) P=0.034 [315].

Data on non-communicable diseases remain scare in the developing countries. The spectrum of these morbidly is wide and includes but not limited to: obesity, diabetes, oncological problems, respiratory diseases including asthma, genetic disorders including sickle cell anaemia (SCA). Globally, however, over one million deaths were registered in children and teens in the year 2002 [316]. This trend is worrisome especial that over 25% of obese adolescents have signs of diabetes by age 15 [316]. Over 90% of the 1 million children born each year with congenital heart disease (CHD) do not access appropriate medical care. Tobacco smoke on either active or passive smokers is responsible for increased incidence of paediatric asthma, otitis, and respiratory infections. Other causes of non-communicable morbidity and mortality in children include: mental health disorders, road traffic accidents, and domestic accidents and poisoning [316]. Survival among children with non-communicable diseases is much lower in resource-limited settings compared to well resourced countries [316].

Delay in seeking appropriate treatment has been implicated in progression of disease and poor outcomes. Rutebemberwa *et al* studied factors responsible for delay seeking treatment outside home settings for children under 5 years of age [317]. They found that households likely to delay seeking treatment were of the lowest socio-economic quintile OR (95% CI) 1.45 (1.06–1.97) or children presenting with clinical pallor OR (95% CI) 1.58 (1.10–2.25). However, they also identified positive practices including: those likely to seek treatment early, for instance, children who sought treatment from drug shops OR (95% CI) 0.70 (0.59–0.84) and community medicine distributors (CMDs) OR (95% CI) 0.33 (0.15–0.74). Moreover, early healthcare seeking behaviour was noted among children who presented

with severity features such as fast breathing OR (95% CI) 0.75 (0.60–0.87) and hyperpyrexia requiring tepid sponging at home OR (96% CI) 0.43 (0.27–0.68) [317]. Short distance to health provider also favoured early healthcare seeking behaviours OR (95% CI) 0.72 (0.60–0.87) [317].

3.3. Approach to the ward surveillance study

While recognizing the limitations of both retrospective and prospective hospital-based surveillance of disease, I adopted a prospective method and embraced a paediatric admission record (PAR) used in Kenya [318], with modification on some sections including laboratory investigations, diagnosis and outcomes.

3.3.1. Aims and Objectives

In this chapter my aims are:

1. To describe the patterns of paediatric admissions at Mbale Regional Referral Hospital
2. To describe the clinical features of paediatric patients admitted to Mbale Regional Referral Hospital
3. To describe the burden of malaria and DUS in relation to other admissions.

3.3.2. Materials and methods

Detailed section on materials and methods has been covered in Chapter 2 section 2.2. However, I conducted a prospective descriptive paediatric admissions ward surveillance study (WSS) between 1st May 2011 and 30th April 2012. Included were all children aged 0 - 144 months, presenting for care at the PACU from 8:00am to

5:00pm on Monday through Friday. Excluded were children who presented beyond 5:00pm and before 8:00am Monday through Friday and those who presented on Saturdays and Sundays. The purpose of the WSS was to describe the clinical features, disease burden, outcome and their temporal relationships with seasons among children admitted to the PACU. Patient admission data was captured using a standardised proforma; the paediatric admissions record (PAR), which included details on patient sociodemographic information, presenting history, examination findings and diagnoses.

Blood samples were taken for blood slide for malaria (microscopy of Giemsa-stained thick and thin blood films for identification and typing of malaria parasites) as earlier described by other researchers [233]. This was done as routine investigation on all children aged >1 month presenting with fever. Lactate estimation (Lactate Pro, ARKRAY Factory, Shiga, Japan) was done on children with clinical features of severe malaria, haemoglobin estimation (HemoCue Hb 301 system, Ängelholm Sweden) was done on all children with clinical features of anaemia (clinical pallor), while HIV test (Determine HIV-1/2 strips, Alere Medical Co. Ltd, www.determinetest.com) was routinely done on all children admitted as part of hospital policy. Other diagnostic tests and investigations where done by attending clinicians as deemed necessary for diagnosis and monitoring of response to treatment.

Underarm (axillary) temperature was measured using a digital thermometer (OMROn, www.omron-healthcare.com). The history of the presenting illness was

taken from the parent or guardian. These and other clinical findings were captured on PAR and the data entered into the database.

Table 3.2. Definitions of common diagnoses

Term	Definition
Anaemia	This is a decrease in the amount of red blood cells (RBCs) or hemoglobin (Hb) in the blood. It was categorised as moderate anemia when Hb was 6 - 9g/dL, severe anaemia when Hb was <5g/dL or profound anaemia when Hb was <4g/dL.
Clinical shock	Fever or hypothermia and clinical evidence of impaired perfusion (capillary refill time (CRT) >2 seconds or lower limb temperature gradient or weak radial pulse volume or severe tachycardia) as we described previously [319].
Coma	A state of unconsciousness where a patient was unresponsive to stimuli.
Dark urine*	Coca-Cola or tea coloured urine, which on HCC scale was in the range of 5 - 10.
Diarrhoea	Passage of three or more loose stool motions in 24 hours and dysentery if the diarrhoea was bloody.
Impaired consciousness	Measured using AVPU scale was defined when AVPU=<A
Malaria	Patient with a positive slide for malaria
Neonatal sepsis	Child <28 days of age with any risk signs: poor feeding, convulsions, poor limb movements, fast breathing, granting, fever (>38^{o}C) or hypothermia <36^{o}C, deep jaundice, severe abdominal distention, skin infections, budging fontanelle and red peri-umbilical area or draining pus
Pneumonia	Cough or difficult breathing plus fast breathing (>60/min for age <1 month, ≥50/min for ages 2 - 11 months, ≥40/min for ages 1 - 5 years) or lower chest indrawing with or

	without crackles on chest auscultation
Prostration	Generalized weakness so that the patient is unable to walk or sit up without assistance [1].
Pyogenic meningitis	A fever or history of fever, seizures, meningeal signs, impaired consciousness and confirmatory CSF analysis
Recurrent convulsions	Two or more convulsions in 24 hours preceding admission
Respiratory distress	Increased work of breathing (deep acidotic breathing or chest indrawing)
Severe malnutrition	Was the mid upper arm circumference (MUAC) <11.0cm, with clinical classification as marasmus if the patient had visible severe wasting and weight for age ≤60% without pitting oedema, but when pitting oedema was present then marasmic-kwashiorkor was defined. Kwashiorkor was defined as weight for age of between 60 - 80% with symmetrical pitting oedema involving at least the feet in the absence of nephrotic syndrome.

*Note: Unless stated otherwise, all definitions were based on the WHO Pocket Book of Hospital Care for Children, first edition [254]. *modified for this study.*

Data analyses have been described in the methods Chapter 2 (see section 2.8). Ethics permission was obtained from MRHIRC. This ethics committee permitted verbal consent for the WSS because it was based on routinely collected data.

3.4. Results

3.4.1. Characteristics of the overall admissions

In the period May 1st 2011 to April 30th 2012, there were 23,217 total admissions to the PACU of which 10,208 (44.0%) children aged 0 - 144 months were enrolled on to the WSS. The median age was 16.0 months. The male: female ratio was 1.25: the factors for this ratio could not be easily established but may be related to the community health seeking behaviors, a possibility that male children have a lower tolerance to pain and illness compared to female children or this finding may actually be by chance. Figure 3.1.

Figure 3.1. Population pyramid of the WSS

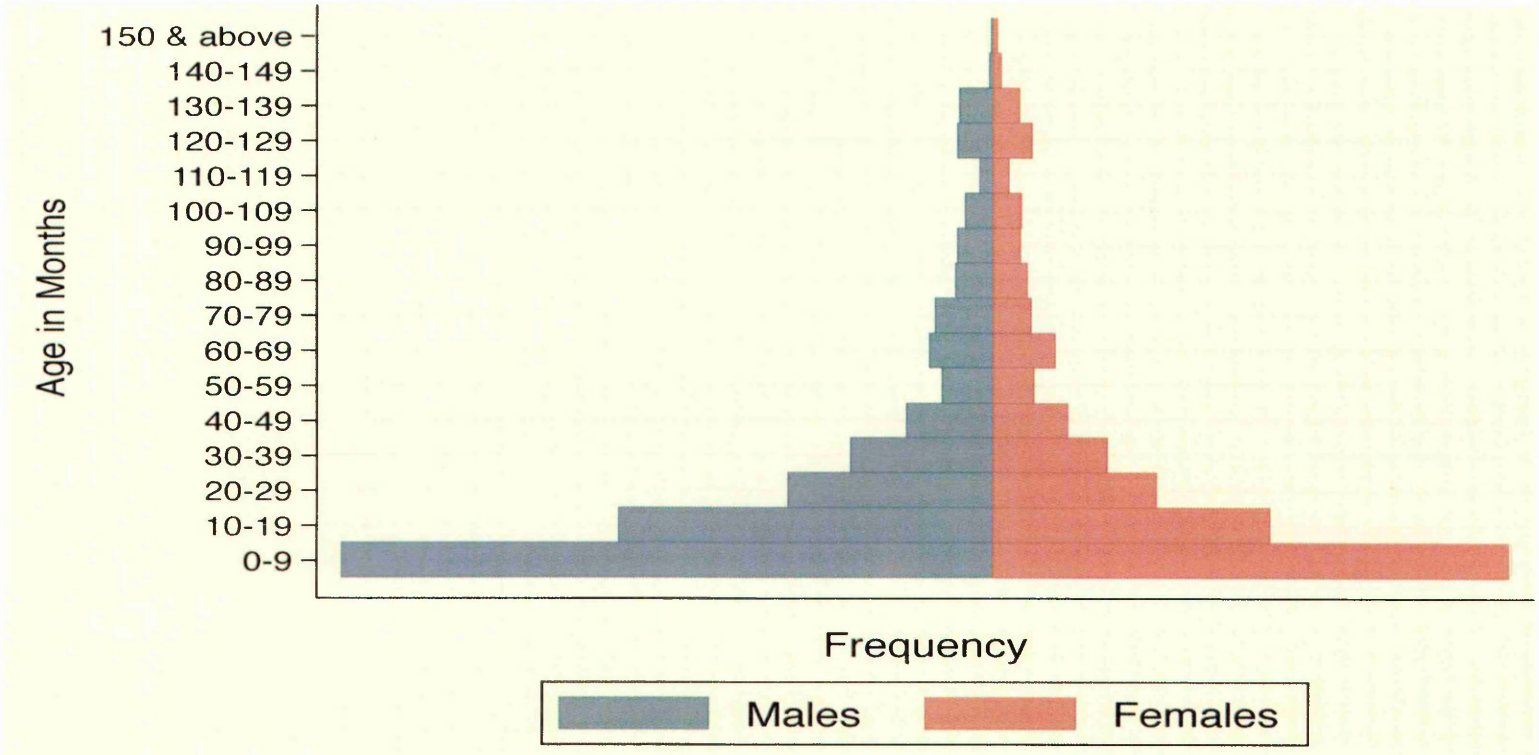


Figure 3.1. This population pyramid shows the age/sex distribution of the study participants in the WSS. A majority of children were aged <59 months. Legend: Blue = males and maroon = females.

The common symptoms included: fever 8,784 (86.1%), followed by cough 7,004 (68.6%), vomiting 5,173 (50.7%), diarrhea 3,395 (33.3%), convulsions 1.132 (11.1%) and history of dark urine 1,087 (10.7%) of whom 666 (61.3) had previous history of dark urine. Patients who had ever had a blood transfusion accounted for 1,331 (13.0%); indicating anaemia with possible recurrences is a common condition in this population (Figure 3.2).

Figure 3.2. Symptoms among 10,208 patients in the WSS

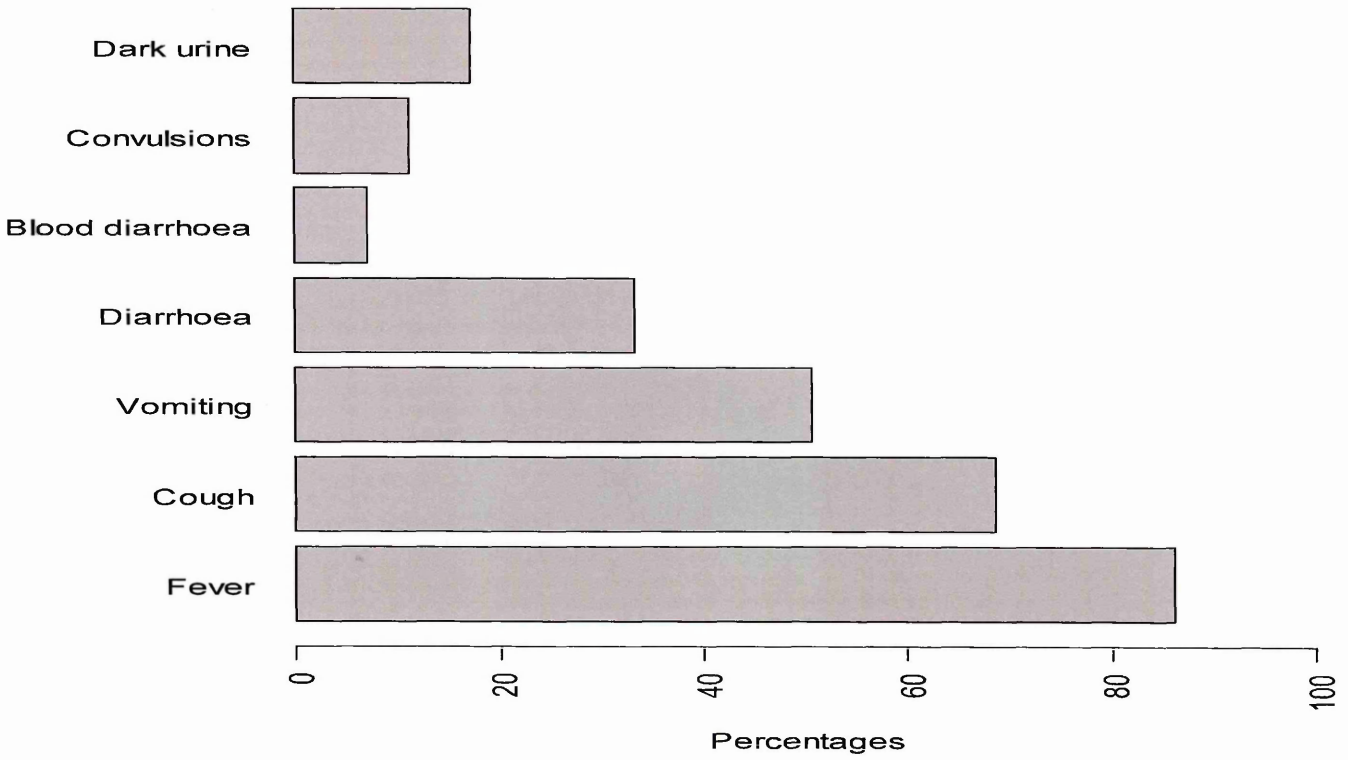


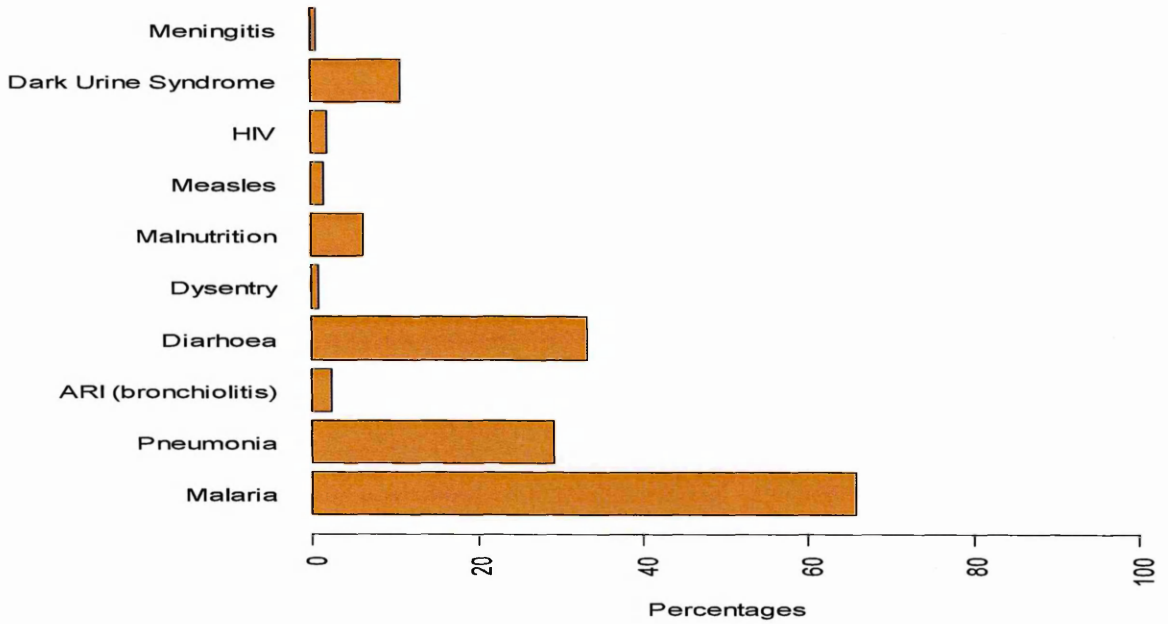
Figure 3.2. Clinical symptoms among 10,208 children in the WSS: in the order of frequency the top 5 most common symptoms at admission were fever, cough, vomiting, diarrhoea and dark urine.

Among the signs, it was not surprising to note features that confirmed the presence or complications of the listed symptoms above. These included pyrexia (>37.5^{o}C) 3,669 (35.9%), pallor; 3,290 (32.2%), respiratory distress; 1,968 (19.3%), severe tachycardia (age graded); 1,762 (17.3%) and capillary refill time >2seconds; 1,499 (14.7%).

In total 6,714 (65.8%) children had a positive slide for malaria of which 662 (~10.0%) had severe malaria as a single cause of admission. The 10% proportion of cases with severe malaria among total malaria admissions was consistent with previous data from malaria endemic areas [1]. Anaemia (Hb <10g/dL) was common 2,020/10,208 (20.0%) of whom severe anaemia (Hb <5g/dL) accounted for 1,655/2,020 (81.9%) and moderate anaemia (Hb 6 - 10g/dL) was among 365/2,020 (18.1%). These were similar to reports on the prevalence of anaemia in areas endemic of malaria in the SSA [109, 110]. HIV positivity rate was low 201/10,208 (2.0%) among the admissions compared to the background overall prevalence of 5.3% in the eastern region (2011 Uganda AIDS indicator survey). This could have been due to positive effect of effective and efficient PMTCT programme which now targeting elimination of mother to child transmission of HIV (eMTCT). However, I could not exclude the negative effect of mandatory HIV testing which was in place at the time of this study where parents with HIV infection could have feared to come for treatment knowing their children would undertake an HIV test. Urinalysis was indicative of UTI in 34/10,208 (0.3%) of the children with dysuria. This was low because of the difficulty catching urine in children, prior antibiotic exposure and the clinician low index of suspicion of UTI in children.

In order of frequency, the top five diagnoses included: malaria 6,714 (65.8%), diarrhoea 3,395 (33.3%), pneumonia 2,970 (29.1%), DUS (history of passing dark/Coca-Cola coloured urine in the present illness) 1,087 (10.7%) and malnutrition (all forms) 541 (5.3%) Figure 3.3.

Figure 3.3. Clinical diagnoses among 10,208 children in the PACU during WSS



Standard definitions of clinical diagnoses were used. Except for Dark Urine Syndrome, all other clinical definitions were based on the WHO Pocket Book of Hospital Care for Children (The Blue Book, as it is commonly referred) [254].

On the outcome, overall 9,553 (93.6%) were discharged home alive while in-hospital mortality occurred among 655 (6.4%) patients.

The data was further reported based on age categorization into neonates (<1 month of age) and children 1 - 143 months for ease of comparison with existing literature. A detailed analysis of symptoms, signs, laboratory features and outcomes based on these categories are presented below:

3.4.2. Sociodemographic features among neonates

The proportion of children aged <1 month 941/9,551 (9.9%) was high. The male (508) to female (408) ratio was 1.25:1. The neonatal case fatality rate (CFR) was high 124/941 (13.2%).

3.4.3. Clinical features among the neonates

Of the 941 infants <1 month of age, the frequent symptoms and their association with mortality were fever 847 (90.0%) with a case fatality rate (CFR) of 114/847 (13.5%) *P*=0.0106, cough 619/941 (65.8%) with CFR of 92/619 (14.8%) *P*=0.0029, vomiting 493/941 (52.4%) with CRF of 82/493 (16.6%) *P*=0.0001. Diarrhoea 322/941 (34.2%) with CFR 39/322 (12.1%) but was not significantly associated with mortality *P*=0.7749. Moreover, bloody diarrhoea with a prevalence of 7/941 (0.7%) was the least common symptom, but among these cases it was surprising that no mortality was associated with it. Convulsions generally are known to be common in neonates and in this study population 95/941 (10.1%) had convulsions a symptom that registered the highest CFR recorded 24/95 (25.3%), *P*=0.0001. However, convulsions lasting more than 30 minutes were infrequent 15 (1.6%) but

had a significantly high CFR 2/15 (12.5%), though its association with mortality was not statistically significant P=0.9255. Table 3.3.

Table 3.3. Symptoms among 941 neonates presenting to the PACU at Mbale RRH

Category	N (%)	Case fatality (With feature)	Case fatality (No feature)	Odds ratio (95% CI)	*P-value**
Patients	941/9,551	124 (13.2)			
Fever	847 (90.0)	114/847 (13.5)	4/94 (4.2)	3.49 (1.26 - 9.71)	0.0106
Cough	619 (65.8)	92/619 (14.8)	26/322 (8.1)	1.98 (1.26 - 3.14)	0.0029
Cough > 3weeks	14 (1.5)	0/14 (0.0)	118/927 (12.7)	-	0.1535
Vomiting	493 (52.4)	82/493 (16.6)	37/448 (8.0)	2.28 (1.51 - 3.46)	0.0001
Diarrhoea	322 (34.2)	39/322 (12.1)	79/619 (12.7)	0.94 (0.63 - 1.42)	0.7749
Bloody diarrhea	7 (0.7)	-	118/934 (12.6)	-	0.3146
Convulsions	95 (10.1)	24/95 (25.3)	94/846 (11.1)	2.70 (1.62 - 4.50)	0.0001
Convulsions >30min	15 (1.6)	2/15 (13.3)	116/926 (12.5)	1.07 (0.24 - 4.82)	0.9255

Table indicating common and rare symptoms among children aged <1 month.

Legend: *N = Number, (percentage), CI = Confidence Interval, * from x^2 values.*

The common signs among neonates varied widely within and between body systems. Pyrexia (>37.5^{O}C) was in 202/355 (56.9%) with a CFR of 31/202 (15.3%) and associated with mortality *P*=0.0184, pallor was noted in 478/941 (50.8%) with CFR of 75/478 (15.7%) with significant association with mortality *P*=0.0030, clinical jaundice was documented in 259/941 (27.5%) with CFR of 46/259 (17.8%) with poor mortality outcomes *P*=0.0029, increased capillary refill time (>2seconds) was found in 216/941 (22.9%) with CFR of 16/216 (7.4%) and respiratory distress in 144/941 (15.3%) and CFR of 44/144 (30.6%). Conversely, rare manifestations included, coma 5/941 (0.53%) with CFR of 1/5 (20.0%) and wheezing 6/941 (0.6%) with CFR of 0 (0.0%). Surprisingly during this study period no neonate had cyanosis. Table 3.4.

Table 3.4. Clinical signs among 941 neonates

Category	N (%)	Case fatality (Feature)	Case fatality (No feature)	OR (95% CI)	*P-value**
Patients	941/9,551	124 (13.2)			
General features					
Pyrexia (>37.50C)	202/355 (56.9)	31/202 (15.3)	11/153 (7.1)	2.34 (1.13 - 4.82)	0.0184
Pallor	478 (50.8)	75/478 (15.7)	43/463 (9.3)	1.82 (1.22 - 2.71)	0.0030
Clinical Jaundice	259 (27.5)	46/259 (17.8)	72/682 (10.5)	1.83 (1.22 - 2.73)	0.0029
Oral thrush	12 (1.3)	1/12 (8.3)	117 /929 (12.6)	0.63 (0.08 - 4.93)	0.6579
Airway/respiratory					
Hypoxia	58 (6.2)	14/58 (24.1)	104/883 (11.7)	2.38(1.26 - 4.49)	0.0059
Respiratory Distress	144 (15.3)	33/144 (22.9)	85/797 (10.6)	2.49 (1.58 - 3.90)	<0.0001
Wheeze	6 (0.8)	-	100/776 (12.8)		0.3464
Indrawing	21 (2.4)	6/21 (28.5)	111/874 (12.7)	2.74 (1.04 - 7.23)	0.0330
Grunting	66 (7.0)	16/66 (24.2)	102/875 (11.6)	2.42 (1.33 - 4.42)	0.0029

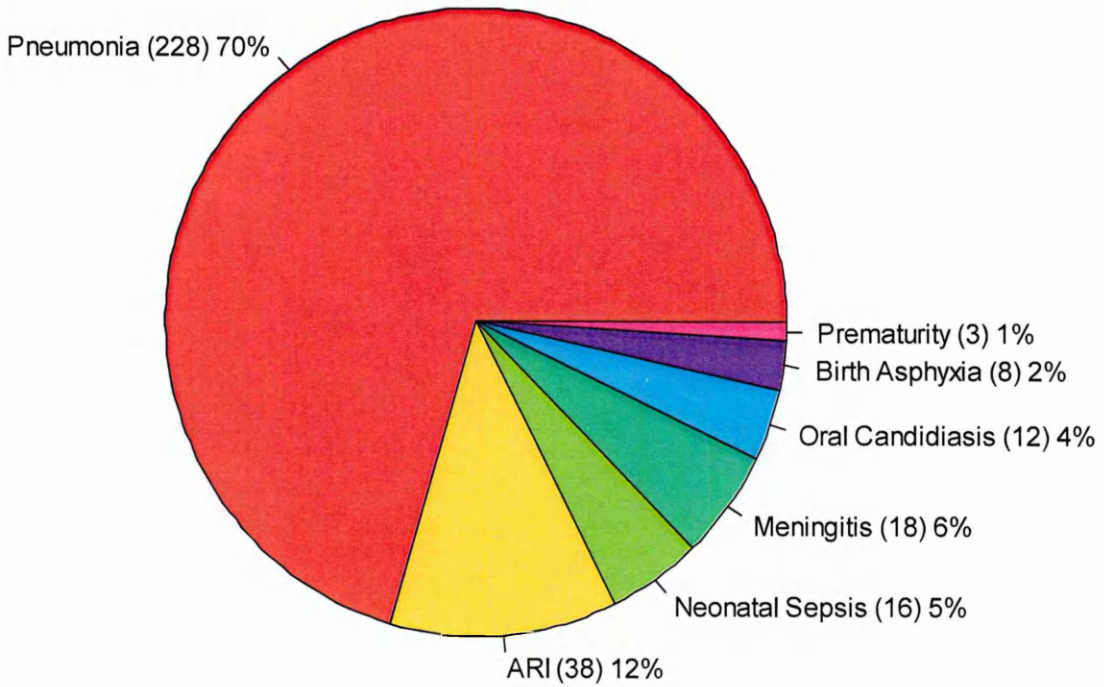
Neurological					
Impaired consciousness	140 (14.9)	25/140 (17.8)	93/801 (11.6)	1.65 (1.02 - 2.68)	0.0395
Prostration	110 (11.7)	25/110 (22.7)	93/831 (11.1)	2.33 (1.42 - 3.83)	0.0006
Coma	5 (0.53)	4/5 (80.0)	114/936 (12.2)	28.84 (3.19 - 260.3)	<0.0001
Cardiovascular/hydration					
Severe tachycardia	57 (6.1)	9/57 (15.8)	109/884 (12.3)	1.33 (0.63 - 2.79)	0.4447
Temp 0C gradient	105 (11.2)	21/105 (20.0)	97/836 (11.6)	1.90 (1.12 - 3.21)	0.0143
CRT >2s	216 (22.9)	36/216 (16.7)	82/725 (11.3)	1.56 (1.02 -2.39)	0.0369
Sunken eyes	133 (14.1)	222/133 (16.5)	96/808 (11.8)	1.46 (0.88 - 2.43)	0.1326
Weak pulse	42 (4.5)	10/42 (23.8)	108/899 (12.0)	2.29 (1.09 - 4.78)	0.0241
Severe dehydration	78 (8.3)	17/78 (21.8)	101/863 (11.7)	2.10 (1.18 - 3.74)	0.0100

Legend: *N = Number, (percentage), CI = Confidence Interval, * from x^2 values, OR= Odds ratio, CRT= Capillary refill time*

Among the laboratory features, severe anaemia (Hb <5g/dL) was found in 246/941 (26.1%); mostly perinatal anaemia often from bleeding, with CFR of 8/246 (3.3%) while moderate anaemia (Hb 6 - 9g/dL) was found among 54/941 (5.7%) with CFR of 1/54 (1.9%).

Figure 3.4 below shows admission diagnoses made for 941 neonatal admissions during the WSS. Pneumonia was the commonest diagnosis in this age group with 228/941 (24.2%) and CFR of 36/228 (15.8%).

Figure 3.4. Diagnoses among neonates in the WSS



Legend: *Diagnosis (number) percentage.*

3.4.4. Socio demographic characteristics for children aged >1 months of age

Children aged >1 months comprised of 8,610/9,551 (90.1%), the median age was 16.0 months with male to female ratio of 1.25:1. The minor variation in gender ratio was possibly due to differences in community health seeking behaviours and may not necessarily reflect the gender demographic profile of the population in Eastern Uganda. But the mortality rate in this group was low 321/8,610 (3.7%).

3.4.5. Clinical features for children aged >1 months of age

A history of febrile illness was present in the vast majority of admissions; however recorded pyrexia or hypothermia was only seen in 7,592/8,610 (88.2%). Respiratory symptoms were very common; yet only 560/8,610 (6.5%) had hypoxia, 1,050/8,610 (12.2%) had chest indrawing and 1,092/8,610 (12.7%) had grunting; signs consistent with the WHO definition of very severe pneumonia. Cyanosis was uncommon but associated with high CRF 3/33 (9.1%), Wheeze (putative Asthma) was very uncommon. The diagnosis of 'respiratory distress', defined as increased work of breathing (deep acidotic breathing or chest indrawing) had a poor prognosis relative to indrawing alone; suggesting that many of the children diagnosed with respiratory distress had metabolic acidosis since difficulty breathing is a clinical sign of this and thus accounting for the poorer outcome.

Of note was the high frequency of vomiting 4,556/8,610 (52.9%) and diarrhoea 3,002/8,610 (34.9%) but fewer children 64/8,610 (0.7%) had bloody diarrhea; the numbers with severe dehydration or sunken eyes alone was considerably fewer,

admission criteria for intravenous rehydration suggesting that these signs were frequent in non-gastroenteritis illness such as malaria

Neurological presentations similarly complicated a range of admission diagnosis: coma was uncommon though was associated with a high CFR 7/55 (12.7%) and a predictor of poor outcome P=0.0002.

Dark urine had a prevalence of 952/8,610 (11.1%) with a CRF of 109/952 (11.4%) and was a predictor of poor outcome P<0.0001. Over 60% of the children with dark urine at admission had previous history of dark urine and this too was significantly associated with mortality (P<0.0001) Table 3.5.

Table 3.5. Symptoms among children aged >1 months presenting to the PACU at Mbale RRH

Category	N (%)	Case fatality (Feature)	Case fatality (No feature)	Odds ratio (95% CI)	*P-value**
Patients	8,610 (90.2)	321 (3.7)			
Fever	7,592 (88.2)	281/7,592 (3.7)	20/1,018(2.3)	1.66 (1.08 - 2.55)	0.0192
Cough	6,142 (71.3)	238/6,142 (3.9)	68/2,468 (2.7)	1.41 (1.07 - 1.85)	0.0135
Cough > 3weeks	172 (2.0)	5/172 (2.9)	299/8,438 (3.5)	0.81 (0.33 - 1.99)	0.6543
Vomiting	4,556 (52.9)	193/4,556 (4.2)	111/4,054 (2.7)	1.57 (1.24 - 1.99)	0.0002
Diarrhoea	3,002 (34.9)	124/3,002 (4.1)	180/5,608 (3.2)	1.29 (1.03 - 1.64)	0.0274
Bloody diarrhea	64 (0.7)	3/64 (4.7)	301/8,546 (3.5)	1.34 (0.42 - 4.32)	0.6148
Convulsions	1,001 (11.6)	60/1,001 (5.9)	244/7,609 (3.2)	1.92 (1.44 - 2.57)	<0.0001
Convulsions >30min	123 (1.4)	4/123 (3.3)	300/8,487 (3.5)	0.92 (0.34 - 2.50)	0.8660
Dark urine in current illness	952 (11.1)	109/952 (11.4)	195/7,658 (2.5)	4.95 (3.87 - 6.32)	<0.0001
Previous history of dark urine	574 (6.7)	61/574 (10.6)	243/8,036 (3.0)	3.81 (2.84 - 5.11)	<0.0001
Previous history of blood transfusion	1,444 (13.3)	78/1,144 (6.8)	226/7,466 (3.0)	2.34 (1.79 - 3.05)	<0.0001

Legend: *N = Number, (percentage), CI = Confidence Interval, * from x^2 values.*

Like the signs for the neonates, the signs among children >1 months of age also varied widely within and between systems. Both pallor 2,682/8,610 (31.2%) and clinical jaundice 1,336/8,610 (15.5%) appeared to be common clinical features, though clinical jaundice would not be expect to be very frequent in children older than one month. Pallor had significant Odd's ratio (95% CI) 2.22 (1.76 - 2.97) for mortality $P<0.0001$.

Many children had signs of shock including temperature gradient 1,250/8,610 (14.5%) and capillary refill time (CRT) >2s, 1,247/8,610 (14.5%). On the other hand, weak pulse was not a very frequent feature 309/8.610 (3.6%). All these three features of shock were markers of poor outcome. Among patients with CRT >2sec 349/1,247 (28.0%) had respiratory distress $P<0.0001$. Of those with weak pulse 185/309 (27.5%) had respiratory distress $P<0.0001$, while those with temperature gradient 495/1,250 (39.6%) had respiratory distress $P<0.0001$. Table 3.6.

A high number of patients 1,444/8,610 (13.3%) presented with history of previous blood transfusion and this was a maker of mortality $P<0.0001$. A high proportion 417/952 (43.8%) of the patients with dark urine also had previous blood transfusion $P<0.0001$.

Table 3.6. Clinical signs among 8,610 children >1 month: prevalence and case fatality rates

Category	N (%)	Case fatality (Feature)	Case fatality (No feature)	Odds ratio (95% CI)	*P-value**
General features					
Pyrexia (>37.5°C)	3,063/5,630 (54.4)	126/3,063 (4.1)	77/2,569 (3.0)	1.38 (1.04 - 1.85)	0.0252
Pallor	2,682 (31.2)	150/2,682 (5.6)	154/5,928 (2.6)	2.22 (1.76 - 2.97)	<0.0001
Clinical Jaundice	1,336 (15.5)	87/1,336 (6.5)	217/7,274 (2.9)	2.26 (1.75 - 2.93)	<0.0001
Oral thrush	90 (1.1)	3/90 (3.3)	301/8,520 (3.5)	0.94 (0.29 - 3.00)	0.9187
Airway/respiratory					
Hypoxia	560 (6.5)	31/560 (5.5)	273/8,050 (3.4)	1.67 (1.14 - 2.44)	0.0078
Respiratory Distress	1,698 (19.7)	81/1,698 (4.7)	227/6,912 (3.2)	1.50 (1.16 - 1.94)	0.0020
Wheeze	81 (1.2)	4/81 (4.9)	248/6,941 (3.5)	1.40 (0.51 - 3.86)	0.5113
Indrawing	1,050 (12.2)	36/1,050 (3.4)	263/7,249 (3.6)	0.94 (0.66 - 1.34)	0.7458
Grunting	1,092 (12.7)	54/1,092 (4.9)	250/7,518 (3.3)	1.51 (1.12 - 2.04)	0.0067
Cyanosis	33 (0.5)	3/33 (9.1)	265/7,343 (3.6)	2.67 (0.81 - 8.81)	0.0931

Neurological					
Impaired consciousness	457 (5.3)	31/457 (6.8)	273/8,153 (3.4)	2.10 (1.43 - 3.08)	0.0001
Prostration	800 (9.3)	60/800 (7.5)	244/7,810 (3.1)	2.51 (1.87 - 3.36)	<0.0001
Coma	55 (0.6)	7/55 (12.7)	298/8,555 (3.5)	4.05 (1.82 - 9.04)	0.0002
Cardiovascular/hydration					
Severe tachycardia	1,670 (19.4)	66/1,670 (3.9)	238/6,940 (3.4)	1.15 (0.88 - 1.53)	0.2988
Temp ^{O}C gradient	1,250 (14.5)	69/1,250 (5.5)	235/7,360 (3.2)	1.77 (1.34 - 2.33)	<0.0001
CRT >2s	1,247 (14.5)	63/1,247 (5.0)	241/7,363 (3.3)	1.57 (1.18 - 2.08)	0.0016
Sunken eyes	974 (11.3)	45/974 (4.6)	259/7,636 (3.4)	1.37 (0.99 - 1.91)	0.0505
Weak pulse	309 (3.6)	19/309 (6.2)	285/8,301 (3.4)	1.84 (1.14 - 2.97)	0.0111
Severe dehydration	668 (7.8)	33/668 (4.9)	271/7,942 (3.4)	1.47 (1.02 - 2.13)	0.0399

Legend: *N = Number, (percentage), CI = Confidence Interval, * from x^2 values.*

In the laboratory findings the main features were malaria 6,576/8,610 (76.4%) and severe anaemia (Hb <5g/dL) 1,374/8,610 (15.9%) both of which were associated with mortality Table 3.7.

Table 3.7. Laboratory findings on malaria and haemoglobin tests

Category	N (%)	Case fatality (Features)	Case fatality (No features)	Odds ratio (95% CI)	*P-value*
Malaria slide positive	6,576 (76.4)	251/6,576 (3.8)	53/2,034 (2.6)	1.48 (1.09 - 2.00)	0.0097
Severe anaemia (Hb <5g/dL)	1,374 (15.9)	84/1,374 (6.1)	220/7,236 (3.0)	2.07 (1.60 - 2.69)	<0.0001
Moderate anaemia (Hb 6 – 9g/dL)	305 (3.5)	12/305 (3.9)	292/8,305 (3.5)	1.12 (0.62 - 2.03)	0.6973

Table 3.7. *Indicating high prevalence of malaria in children >1 months, this is expected in malaria high transmission settings where children <5 years bear the biggest burden of malaria. The prevalence of severe anaemia (Hb <5g/dL) requiring blood transfusion was also high but within the range reported for SSA.*

Legend: *N = Number, (percentage).*

The common diagnoses were malaria, diarrhoea, pneumonia, dark urine and ARI. It was surprising to find that in this study population the overall prevalence of malnutrition was low, but even more surprising that mortality was low among patients with malaria and pneumonia, Table 3.8.

Table 3.8. Spectrum of clinical diagnoses and outcome among 8,610 children in the WSS

Characteristic	N (%)	Case Fatality Rate, N (%)
Number (%)	8,610 (100.0)	321/8,610 (3.7)
Pneumonia	2,617 (30.4)	94/2,617 (3.6)
ARI (bronchiolitis)	187 (2.2)	3/187 (1.6)
Malaria	6,576 (76.4)	253/6576 (3.8)
Diarrhoea	3,002 (34.8)	125/3,002 (4.2)
Dysentery (bloody diarrhea)	64 (0.7)	3/64 (4.7)
Kwashiorkor	201 (2.3)	6/201 (2.9)
Marasmus	160 (1.9)	9/160 (5.6)
Marasmic-kwashiorkor	91 (1.1)	1/91 (1.1)
Measles	139 (1.6)	1/139 (0.7)
HIV	186 (2.2)	6/186 (3.2)
UTI	30 (0.4)	1/30 (3.3)
Dark urine syndrome	952 (11.1)	109/952 (11.4)
Oral candidiasis	90 (1.1)	3/90 (3.3)

***Table** 3.8. Indicating diagnoses with severe malnutrition having high case fatality rates. Patients often had more than one diagnosis Legend: N = Number, (percentage).*

The monthly admissions varied widely with two-peak admissions registered in June - August and December - February, these were also the peak rainy seasons for the eastern region during the study period. These rainy seasons corresponded to the peak malaria, severe malaria and dark urine syndrome admissions; Table 3.9.

Table 3.9. Trends in the monthly admissions during WSS

Month	Number Admitted	Malaria, N (%)	SMS, N (%)	PRODUES, N (%)	Mortality, N (%)
May	727	536 (73.7)	63 (8.7)	95 (13.1)	37 (5.1)
June	626	326 (52.1)	36 (5.8)	89 (14.2)	26 (4.2)
July	1,574	1,020 (64.8)	102 (6.5)	195 (12.4)	76 (4.8)
August	982	665 (67.7)	70 (7.1)	96 (9.8)	56 (5.7)
September	731	511 (69.9)	83 (11.4)	89 (12.2)	54 (7.4)
October	652	420 (64.4)	85 (13.0)	63 (9.7)	67 (10.3)
November	440	306 (69.5)	40 (9.1)	45 (10.2)	30 (6.8)
December	663	444 (66.9)	63 (9.5)	75 (11.3)	65 (9.8)
January	1,033	670 (64.9)	22 (2.1)	108 (10.5)	96 (9.3)
February	994	641 (64.5)	23 (2.3)	68 (6.8)	54 (5.4)
March	947	629 (66.4)	35 (3.7)	79 (8.3)	53 (5.6)
April	839	546 (65.1)	40 (4.8)	85 (10.1)	41 (4.9)

Legend: *N = number, percentage in brackets, SMS = Severe malaria surveillance, inclusion criteria in section 2.3, PRODUES = Prospective dark urine epidemiological study, inclusion criteria in section 2.4.*

Figure 3.5. Trends in admission patterns over the 12-month study period

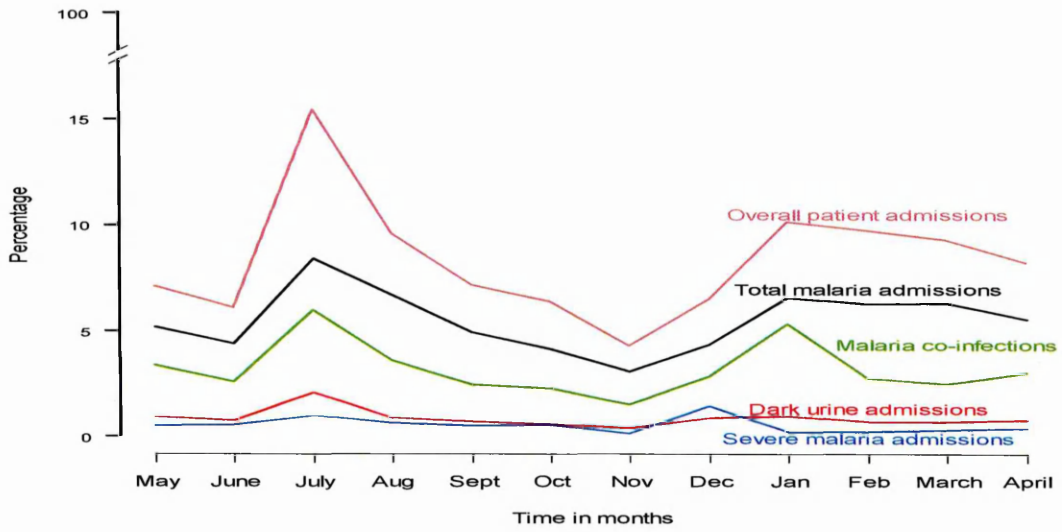


Figure 3.5*. Indicating total admissions in relation to malaria and dark urine syndrome admissions and seasons during the 12-months survey at the PACU of Mbale RRH.*

Legend: the first 3 letters of the name of the month represent each month.

3.5 Discussion

This was the first hospital-based surveillance to describe the clinical presentation, diagnoses and outcomes among children admitted to the PACU at Mbale RRH in Eastern Uganda. The overall median age in this study population was 16.0 months and as expected the majority (87.2%) of the children were under 5 years of age. The male to female ratio was 1.25: 1; the differences in the ratio are possibly due to the differences in the community health seeking behaviours, cultural practices that emphasise the importance of male children, low level of tolerance to pain and disease in males compared to female children or may have been purely due to chance. Most children presented with fever 8,784/10,208 (86.1%), cough 7,004/10,208 (68.6%), vomiting 5,173/10,208 (50.7%) and diarrhoea 3,395/10,208 (33.3%); which mirrored the common diagnoses of malaria, pneumonia and diarrhoea. Malaria was a major problem in children especially among <5 year olds, similar to earlier reports by other researchers [10, 35, 220].

Whereas the PACU was designed specifically for paediatric admissions aged 0 - 144 months, the policy at the PACU considers admitting older children up to the age of 18 years for some conditions including sickle cell anaemia, cerebral palsy and severe malnutrition. The first two conditions are not captured in the PAR and sickle cell in particular is a common cause of admission in Eastern Uganda, so the PAR should probably be adapted to including this field in future. The overall in-hospital mortality was 655 (6.4%); similar to one study in Monrovia, Liberia (6.4%) [261] and the series in Kenya hospitals [245] but was much lower than the rates from another study in Liberia (13.1%) [262] and in Zimbabwe (17.8%) [320]. Since most deaths in these settings occurred within 24 hours, it is probable that poor

emergency care accounts for the high mortality. Since most children had malaria and anaemia, delay in treatment as a result of logistical or total lack of supplies may have contributed to high mortality. Though, at this stage this may be difficult to independently verify, it is plausible that delay transfusing a severely anaemic child and instituting appropriate antimalarial treatment for severe malaria potentially contributes to higher mortality. In my study, however, mortality was not uniform across the common demographic age groups. The neonates (<1month) had poor mortality outcomes with a high case fatality rate of 13.2% compared to children >1 month at 3.7%, P=0.0001; indicating that heavy case burden was borne by the neonates compared to older children. Of particular note is that; whereas, the commonest diagnosis in neonates was sepsis, many children in this group presented with diarrhoea and vomiting; features not typically associated with sepsis in this group but may reflect risk associated with the community feeding practices where infants get mixed feeding as early as first month of life.

Malaria 6,714 (65.8%) was the commonest cause of admission. Other than those with severe malaria 662 (~10.0%), the rest of the patients with malaria were admitted with possible co-infections with viral, bacterial or fungal infections; indicating that infectious diseases were the main causes of admissions to this unit. The median age for children with malaria was 12.0 months with age of first attack at 2 months suggesting that the role of maternal protection of infants against malaria in malaria high transmission areas may not be as effective as previously thought.

This study also found that pneumonia was common with one out of every three children suffering from the disease. However it was surprising to find a higher proportion of older children (>1 month) had pneumonia compared to neonates. The factors underlying this observation may be beyond the scope of this study, but it is plausible to think that since the pneumococcal conjugate vaccine (PCV) had not yet been introduced or used in the region by the time of this study, many children were highly susceptible to *S. pneumoniae* as common pneumonia causing organism. Nonetheless, the proportion of mortality in neonates with pneumonia was three fold that in older children suggesting high virulence of bacteria in already vulnerable newborns with poor immunity. However, because the PAR did not provide for the differential diagnosis of conditions such as prematurity of the newborn, meconium aspiration and hypoxic ischemic encephalopathy (HIE), pneumonia may have been over diagnosed. Nonetheless, in Uganda pneumonia in children is ranked second to malaria [290, 298]; and my results are consistent with that trend.

Diarrheal diseases were common in children >1 month of age and may be as a result of a number of factors that cut across the relationships in the epidemiological triad of host, environment and agent. Since a majority of my study participants were aged less than 5years and a greater proportion in this age group were infants, it is likely that infant weaning practices and poor immunity played a larger role in predisposing these children to infections leading to diarrhoea. In addition, a vaccine against rotavirus, a proven public health intervention was not routinely used in Eastern Uganda at the time of this study. Furthermore, most of my study participants came from either rural or slum areas,

environments long known for poor sanitation [302, 307] and hence a risk factor for diarrheal diseases.

Anaemia is a common cause of morbidity in children especially among <5years old. The causes of anaemia are multi-factorial with several co-factors causally related to mortality risk. To improve on the poor severe anaemia outcomes, it is thus likely that complementary treatment approaches will be necessary, including both immediate (transfusion), and longer-term interventions. Malaria still plays an important role in severe anaemia, even if this is less than in previous decades. In Kilifi, Kenya, recent reports of epidemiological transition in malaria transmission has led to a decline in hospital paediatric admissions (including malaria and severe anaemia)[287] and reduced demand for transfusion. However, severe anaemia case fatality over this period remained unchanged at 8-10%[321].

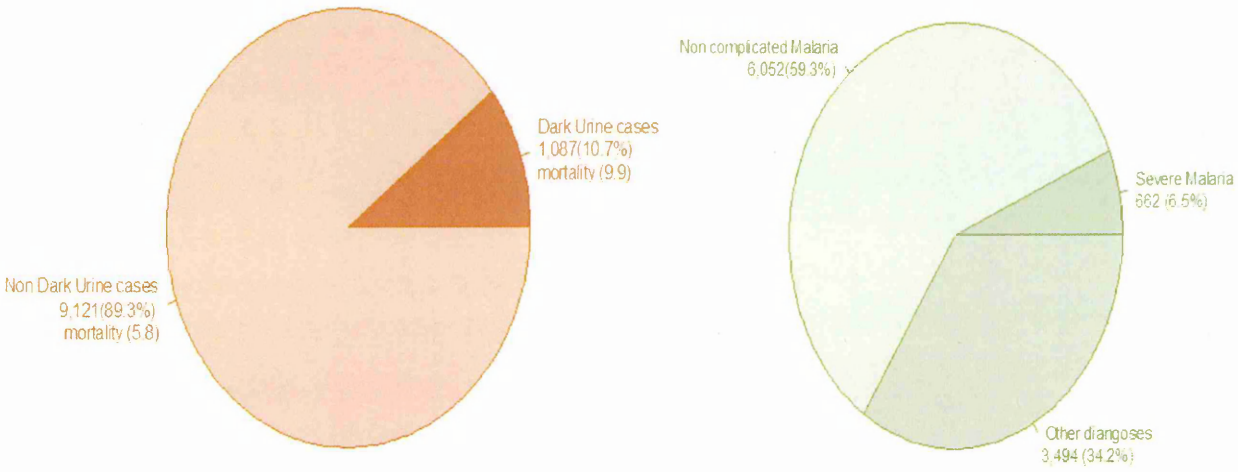
In the only comprehensive case-control study of children hospitalized with severe anaemia in Africa [322], key associations (OR; 95% CI) with severe anaemia were bacteremia (5.3; 2.6-10.9), malaria (2.3; 1.6-3.3), hookworm (4.8; 2.0-11.8), HIV infection (2.0; 1.0-3.8), vitamin A deficiency (2.8; 1.3-5.8) and vitamin B12 deficiency (2.2; 1.4-3.6). Neither iron nor folate deficiencies were associated with mortality, and were less prevalent among cases than controls as iron, folate and anti-helminthics (for any child >1year of age that has not received antihelminths in the last 6 months) are already recommended post-SA-discharge [323].

The prevalence of anaemia of all severities was high in this study similar to that reported in some areas endemic with malaria in the SSA [109, 110]. As expected, there was a strong relationship between severe anaemia and malaria; $P<0.001$.

However, most anaemia was among young children (median age 12.0months); a feature that has been described in malaria endemic areas [8, 113, 118]. I found a strong relationship between severe anaemia with malaria (P<0.001) and moderate anaemia with malaria (P=0.002), similar to findings by earlier researchers [8, 116].

Dark urine based on clinical history as earlier defined by other researchers [183, 190, 324, 325], was also common 1,087 (10.6%) in my study. Studies on aetiology of dark urine in infants point to a haemolytic process due to an inherited genetic abnormality such as G6PD [202]. Though we do not have data to support the role of autoimmune phenomenon in this population, other authors have published its role in older children [326]. I have given a detailed description of DUS in chapters 5 and 6. Based on my surveillance data it was interesting to portray proportions on dark urine phenomenon on one hand and those of malaria on the other to get insight on the burden of the two conditions: severe malaria and DUS, Figure 3.6. I further explored in my thesis severe malaria, Chapters 4, dark urine in chapters 5 and 6.

Figure 3.6. Clinical diagnoses of malaria, dark urine and other diagnoses among 10,208 children in the WSS



The seasonal admission trends of patients with dark urine and malaria were similar, suggesting either similar risk or cause - effect relationship as has been described further in chapter 5.

It was surprising to find low prevalence of severe acute malnutrition (SAM) in my study since the recent Uganda nutrition situation analysis indicated that in Eastern Uganda the prevalence of stunting was 38%, underweight was 16% and wasting 6% [327]. However, my study comprised of a selected group of patients reporting to a regional referral hospital and may not accurately reflect the community prevalence of malnutrition or malnutrition reporting to lower health facilities, which are proximal to the community.

The prevalence of HIV and its case fatality rate were low 182/8,610 (2.1%), despite a general national increase in prevalence of HIV from 6.3% before 2010 to 7.3% in 2011; it is even higher in internally displaced people's camps in the northern part of the country [328]. Nonetheless, in the last five years, there has been a general decrease of mother to child transmission due to the strengthening of PMTCT policy and effective antiretroviral drugs for HIV-positive pregnant mothers and exposed newborns. As the predominant age in my study was <5 years, the PMTCT intervention may have had an impact on paediatric HIV transmission. In the FEAST study (2011) the HIV prevalence was 182/8,610 (4.3%) [159]; though this was a pre-selected group of children presenting with severe illness.

Generalizability of these results need to be done thoughtfully; considering that this study was conducted in a malaria high transmission area, in a regional referral centre and that there were no community data. In addition, diagnosing most conditions based on clinical criteria at admission may have affected accuracy of numbers of patients in each clinical category. I however, ensured that clinical diagnostic accuracy is improved by prior and re-training of the clinical survey team to recognise clinical signs as well as good clinical practice (GCP). There could have been inter-observer variation in reading HCC scale especially between the child's caregiver and the clinician. This and the inter-observer variation between clinicians were both not corrected for, however, both the caregivers and clinicians new the natural normal urine colour. In addition, we used commonly used terms including "Coca-cola" and/or "tea" colour to describe dark urine.

In conclusion, this hospital-based surveillance on admission patterns of disease has revealed that malaria and its complications are common medical problems among children of all age groups above 1 months of age admitted to the PACU at Mbale RRH. Being the first surveillance it has provided a background for future surveillance to monitor temporal relationships among the common illnesses seen at the PACU. The case fatality rates were higher in neonates compared to older children.

CHAPTER 4: Childhood Severe Malaria in Eastern Uganda

4.0. Abstract

The WHO 2010 updated criteria on clinical and laboratory features of severe *P. falciparum* malaria were used in the surveillance of severe childhood malaria in Eastern Uganda. In total 662 children aged 2 - 143 completed months qualified and were recruited during the 12 months prospective study conducted between May 1st 2011 and April 30th 2012. The median age in completed moths (IQR) at admission was 18 (10; 33) and the sex ratio of male to female was 1.4: 1. Respiratory distress 554 (83.7%) was the most common clinical feature, followed by shock 411 (62.1%), clinical jaundice 177 (26.7%), severe anaemia 169 (25.5%), hyperlactataemia 134/660 (20.3%) and DUS 93 (14.0%). The overall mortality was 63 (9.5%). High case fatality rates (CFR) were registered in hypoxia 14/49 (28.6%), impaired consciousness 17/82 (20.7%) and coma 9/52 (17.3%). Respiratory distress was common in dual combinations of clinical features with poor outcomes namely: with shock (345 (52.1%), CFR 6.9%, P=0.0199); with anaemia (151 (22.8%), CFR 17.9%, P<0.001), with impaired consciousness (75 (11.3%), 20.0%; P=0.001); with jaundice (144 (21.0%) CFR 12.3%; P=0.167) and with severe lactataemia (123 (18.6%), CFR 24.4%; P<0.001). Triple combinations of clinical features included: severe anaemia, respiratory distress and impaired consciousness (22 patients (3.0%), CFR 36.4%); another triple combination included severe anaemia, impaired consciousness and severe lactataemia (19 (3.0%); CFR 26.3%) and in the triad of severe anaemia, hypoglycaemia and prostration there were (0.3%) patients and

CFR of 1 (50.0%). Eleven clinical features at univariate analysis were associated with mortality including: pyrexia (>37.5^{o}C), pallor, impaired consciousness, capillary refill time >2seconds, splenomegaly, hepatomegaly, hypoxia, severe anaemia (Hb <5g/dL), severe lactataemia (>5mmol/L), hypoglycaemia (<2.2 mmol/L) and leucocytosis. Multivariate logistic regression analysis identified three features that were independently associated with an increased risk of mortality: hypoxia (P=0.007), severe anaemia (P=0.028) and severe lactataemia (P=0.001).

In conclusion, the common clinical features of childhood severe malaria among patients admitted to the paediatric acute care unit (PACU) at Mbale Regional Referral Hospital (Mbale RRH) were: respiratory distress, shock, clinical jaundice and severe anaemia. Blackwater fever (dark urine syndrome) and clinical jaundice, both of which are rarely reported in other studies, were surprisingly frequent in this study population. Coma was less prevalent in this series compared to other series possibly because of high malaria transmission intensity. Mortality was high in this study with an increased case fatality rate in patients with more than one clinical feature.

4.1. Introduction

Globally, malaria remains a public health problem. It is probable that the descriptions of the clinical features of childhood severe malaria across different geographical, epidemiological and transmission settings in Africa remain incomplete [9, 32, 329]. The last 25-year period has seen more descriptions of clinical features of severe malaria, which have shaped a conceivable regional disease depiction within sub-Saharan Africa (SSA). In the East Africa region, frequently reported features include impaired consciousness, respiratory distress and severe malarial anaemia [8, 58, 65, 330, 331]. In West Africa, data from the far West indicate that the common clinical features include impaired consciousness, hypoglycaemia and lactic acidosis [26, 149], while the clinical descriptions from the mid West region frequently include cerebral malaria, hypoglycaemia, respiratory distress and lactic acidosis [162, 332-334]. Moreover, the clinical characteristics often reported in the mid Southern African areas are similar to those reported in the far West African region including; impaired consciousness, hypoglycaemia and lactic acidosis [23, 335]. However, these data are from few research sites. Furthermore, data appraising the frequencies and distribution of these clinical features within larger sample sizes, non-research settings and serially over time are still lacking. Therefore, additional studies of childhood severe malaria across SSA, both in research and non-research settings, remain desirable for improvement in precision of case recognition, epidemiology and guidance of case management. The detailed review of the current literature on severe malaria has been covered in Chapter 1; however, in the following section I

highlight the neurological complications, haematological markers, metabolic components and general clinical features and risk factors for poor outcomes.

4.1.1. Spectrum and complications of severe malaria

Age has been associated with neurological features depending on transmission intensity. Cerebral malaria has been shown to affect older children in areas with seasonal transmission but in areas with intense transmission young age is still the most affected [336]. Similarly, a study in Uganda indicated that children with cerebral malaria were significantly older (median age = 2.5 years, IQR=1.5-3.9 years) than those without cerebral malaria (median age = 1.7 years, IQR=1.0-2.9 years), $P<0.0001$ [10]. Neurological manifestations are associated with poor outcomes in severe malaria compared to children without such features. For instance, in the AQUAMAT study, mortality in children with profound coma (BCS ≤2) accounted for a higher case fatality rate, 347/1,645 (21.1%) compared to those without 178/3,777 (5.0%), $P<0.001$. In addition, mortality among children with convulsions was higher 242/1,692 (14.3%) compared to those without 285/3,734 (8.0%), $P<0.001$. [61]. Among survivors, neurological sequelae are reported in 1 - 10% with adults in the lower and children in the upper limits. The geneses of sequelae are often associated with recurrent convulsions, hypoglycaemia and acidosis [37, 39].

Severe anaemia remains common but its aetiology until recently was not well understood. Newton *et al* did an assessment on severe anaemia among children admitted to the Kilifi District Hospital in the coastal Kenya. In the two-year period (1 September 1989 - 31 August 1991), 2,677 children were admitted but malaria

and Hb assessments were done on 2,340 children who were older than one month [132]. Malaria was diagnosed in 676/2,340 (28.8%) of whom 141 (20.9%) also had severe anaemia (Hb <50g/L). Furthermore, these authors characterized anaemia in 101 children and found malaria 46/101 (46.0%) was the most common cause of severe anaemia. The authors noted that the other causes of severe anaemia were iron deficiency especially in association with hookworm infection, but also gastrointestinal bleeding, marasmus and sickle cell disease [132]. It is worthy noting that the primary cause of anaemia was not identified in a substantial proportion of children 23/101 (23%) [132]. This points to the fact that the causes of anaemia in severe malaria are multi-factorial. This is confirmed in a case-control study of children hospitalized with severe anaemia in Africa [322]. These authors noted the key associations with SA were bacteremia (OR=5.3; 95% CI 2.6-10.9), malaria (2.3; 1.6-3.3), hookworm (4.8; 2.0-11.8), HIV infection (2.0; 1.0-3.8), vitamin A deficiency (2.8; 1.3-5.8) and vitamin B12 deficiency (2.2; 1.4-3.6). Cserti-Gazdewich *et al* in their study comparing children with severe and non-severe malaria in Kampala Uganda, observed that thrombocytopaenia was a marker of severity and together with leucocytosis was a contributor to a poor outcome in childhood severe malaria [10]. Zeidan *et al* reported convulsions as other risk factors for a poor outcome in a study conducted in Sudan [220]. Both Ajetunmobi *et al* in Nigeria [180] and Kunuanunua *et al* in the DRC [179] found that renal failure in severe malaria was associated with blackwater fever and that this phenomenon disproportionately affected older children. Other studies have looked at the overlap between various clinical features of severity. For instance, Maitland *et al* found that acidosis in severe malaria was associated with both

impaired consciousness and shock [50]. Similarly, English *et al* described respiratory distress as a marker of severe metabolic acidosis [28], while Idro *et al* described a strong association between coma and severe anaemia [123].

4.1.2. Risk factors for poor outcome

The risk and prognostic factors for poor outcomes vary depending on transmission intensity, age and geographic location. In Africa, transmission intensity appears to determine the burden and clinical features of malaria [110]. Studies done in moderate-to-high transmission areas indicate that young age, especially below 5 years, is associated with a higher risk for severe malaria [98, 221] and even a younger mean age in high transmission areas [22]. These data indicate that age, immunity and background transmission intensity are host and environmental determinants of risk to severe malaria. In childhood severe malaria, clinical features with poor outcomes among children include impaired consciousness or coma [104, 123, 127]. In corroboration, in a postmortem study of 90 deaths in Malawian children with clinical diagnosis of cerebral malaria, White *et al* reported that 64 (71.1%) of the autopsies were conclusive of cerebral malaria [63]. The case fatality in cerebral malaria is generally high with most data reporting case fatality rates in the range 10 - 41% [24, 222, 223], and disproportionally affecting children in rural compared to urban settings [224].

The outcome is even poorer when associated with acidosis [50]. Other features with poor outcomes include respiratory distress, multiple convulsions and hypoglycaemia [158]. In a recent multicentre randomised trial (AQUAMAT) the five predictors of poor outcome included base deficit, coma score, convulsions,

BUN and chronic illness [61]. In addition, high case fatality rates have been noted in patients with severe hyperkalaemia [158].

4.1.3. Studies on severe malaria in Uganda

In Uganda, descriptive studies of the clinical spectrum of severe malaria have been conducted in the Southwestern, Central and Southeastern part of the country between 2003 and 2006 [22, 24, 225]. In Central Uganda, at Mulago Hospital Kampala; of the 784 children with severe malaria studied at admission, the commonest manifestations were severe anaemia (39.4%), respiratory distress (17.1%), multiple generalized convulsions (13.3%), hypoglycaemia (11.4%) and cerebral malaria (7.2%) [24]. In Western Uganda, at Kabale hospital; 117 consecutive patients with severe malaria were assessed in a 17-month period from 2001 to 2002, of whom only 51 (43.6%) were <5 years of age. Fever, vomiting, and cough were the most frequent symptoms. In the <5 year olds, the commonest manifestations were; prostration (45.1%), respiratory distress (29.4%) and severe anaemia (19.6%), but cerebral malaria was uncommon [225]. As previously referred [22], the median age in months in children with severe malaria was lower with increasing transmission intensity, that is, the higher the transmission intensity the younger the median age of children with severe malaria. In addition, the manifestations varied with transmission intensity, severe anaemia being more common in high transmission areas, while neurological features were frequent in low transmission intensity areas, a finding consistent with other reports from Africa [1]. In moderate transmission areas respiratory distress seems to be the

most frequent feature though there was a slight variation at Mulago between the study done in 2004 and that in 2006 by the same primary author [22, 24]. This study [22], nonetheless, did not comparing the clinical spectrum and outcome.

However, to date there have been no descriptions of childhood severe malaria published from the Elgon zone of Eastern Uganda, where the clinical spectrum is thought to be different from the picture in the rest of country. I speculated that in Eastern Uganda, where malaria transmission is intense and perennial the clinical spectrum of childhood severe malaria may differ from that reported in the current literature. This is because I had witnessed many cases presenting to the hospital with the clinical triad of haemoglobinuria, jaundice and anaemia in children with malaria in this region, which from the other reports in Uganda and other countries appear to be rare, and thus I hypothesized that this phenomenon may be of unique relevance to the populations of Eastern Uganda. The differences in this clinical spectrum from that reported may partly be explained by the epidemiological characteristics of this syndrome, which are dependent on intensity of malaria transmission, location, age, season and temporal relationship.

4.2. Aims and Objectives

In this chapter the following were my objectives:

1. To describe the spectrum of clinical manifestations of childhood severe malaria in Eastern Uganda.

2. To identify the factors which might have association with the development of complications and mortality in childhood severe malaria in this region.

4.3. Materials and methods

I used the materials and methods described in detail in Chapter 2. Briefly, this was a descriptive study at the PACU in Mbale RRH (Chapter 2 section 2.3), in the background of high malaria transmission [229]; (Chapter 2 section 2.1). The study participants included children fulfilling the WHO 2010 clinical definition for severe malaria (Chapter 2; Table 2.1) [1]. Standardised data management processes were adhered to and respective data analyses done (Chapter 2; sections 2.7 and 2.8). Ethical considerations were observed before commencement, during and after the study (Chapter 2; section 2.11).

4.4. Results

4.4.1. Sociodemographic features

Between May 1st 2011 to April 30th 2012, six hundred and sixty two children aged 2–143 completed months of age presented with clinical features consistent with the WHO 2010 criteria for severe childhood malaria [1]. This sample size was considerable in comparison with similar earlier studies elsewhere [8, 11, 25] and in a recent study in Kampala, Central Uganda [10].

All the study participants were residents within the 14 districts of Mbale RRH catchment area. The bulk of admissions, 361 (54.5%) resided in the slums (peri-urban) areas, followed by 228 (34.4%) from the rural areas and only 73 (11.0%) were children from urban areas. Bantu ethnicity 558 (84.3%) was predominant possibly because of the location of the study site. Other ethnicities were Nilohamites with 82 (12.4%), Luo 10 (1.8%), and other minority ethnic groups constituted 9 (1.6%).

The median age was 18 months (IQR 10; 33) and the male: female ratio 1.4:1, with only few children 60/662 (9.1%) in the category >5 years. A high proportion of children came from slums (defined as heavily populated part of peri-urban area inhabited by people of low social economic status) 361/662 (54.5%).

The overall case fatality rate (CRF) was 63/662 (9.5%) and there were no differences in mortality between the two genders, *P*= 0.478. The majority 603 (91.9%) of children studied were under 5 years of age, CRF in this group of 57/603 (9.5%); contributing to 57/63 (90.5%) of all the severe malaria deaths. Within the <5 year olds, there were similar proportions and fatality rates across the age groups: 2 - 11 months [204 (30.8%); case-fatality rate 9.8%] 12 - 23 months [204 (30.8%); case fatality rate 9.8%] and 24 - 59 [194 (29.3%); case fatality rate 8.2%]. Only 60 children (9.1%); case fatality rate 10.0%, were aged above 5 years. However, case fatality rates had a pattern that possibly reflected proximity to healthcare with children in urban centres having the lowest case fatality rates 5/73 (6.8), compared to slums/peri-urban with 32/361 (8.9%) and those from rural areas had the highest case fatality rates of 26/228 (11.4%), (Table 4.1).

4.4.2. General clinical features

The clinical characteristics of severe malaria patients identified through this study are summarized in Table 4.1. Like in other series [8, 225], fever was the most commonly reported symptom 648 (98.0%) at admission with a median duration of 2 days (IQR 1; 3). However, recorded pyrexia (>37.5^{o}C) was found in 411 (62.1%), hyperpyrexia (axillary ≥40.0^{o}C) in 55 (8.3%) and hypothermia (<36.0^{o}C) in 11

(1.7%). Clinical pallor was found in 338 (51.1%), with a case fatality rate of 45/338 (13.3%); while clinical jaundice was surprisingly high, 177 (26.7%), (Table 4.1).

Many children had signs of shock including temperature gradient 251/662 (37.9%) and capillary refill time (CRT) >2sec, 135/662 (20.4%). On the other hand, weak pulse was not a very frequent feature 23/662 (3.5%). Specifically, CRT >2s was marker of poor outcome. Despite many children having diarrhoea, vomiting and features of shock, it was surprising that only 10.4% of the children had sunken eyes, a sign of dehydration.

Severe malaria was also complicated by neurological presentations: coma was less frequent 52/662 (7.9%) but was associated with a high CRF 9/52 (17.3%) and a predictor of poor outcome P=0.046. Dark urine had a prevalence of 93/662 (14.0%) with a high CRF of 12/93 (12.9%).

Table 4.1. Clinical characteristics of 662 children with severe malaria in Eastern Uganda

Variable	N (%)	Case Fatality (%)	OR (95% CI)	*P-value**
Number	662 (100)	63 (9.5)	-	-
Age				
Median age (IQR)	18 (10; 33)	18 (9; 31)	-	<0.001
0 – 11	204 (30.8)	20 (9.8)	-	0.866
12 – 23	204 (30.8)	21 (9.8)	-	0.649
24 – 59	194 (29.3)	16 (8.2)	-	0.474
60 – 143	60 (9.1)	6 (10.0)	-	0.894
Gender				
Male Sex	382 (57.7)	39 (10.2)	1.20 (0.73 – 1.93)	0.478
Female Sex	280 (42.3)	24 (8.6)	-	-
Duration of fever Media (IQR)	2 (1 – 3)	-	-	-
Residence				
Rural	228 (34.4)	26 (11.4)	1.34 (0.83 – 2.15)	0.231
Slums	361 (54.5)	32 (8.9)	0.86 (0.54 – 1.37)	0.531
Urban	73 (11.0)	5 (6.8)	0.69 (0.29 – 1.68)	0.410

Clinical symptoms				
Fever in this illness	648 (98.0)	62 (9.6)	1.34 (0.19 – 8.98)	0.760
Cough	130 (19.6)	9 (6.9)	0.68 (0.34 – 1.34)	0.261
Vomiting	266 (40.2)	24 (9.0)	0.92 (0.56 – 1.48)	0.723
Diarrhoea	238 (36.0)	24 (10.1)	1.10 (0.68 – 1.78)	0.709
Bloody diarrhoea	16 (2.4)	1 (6.3)	0.65 (0.10 – 4.41)	0.652
Convulsions	147 (22.2)	24 (16.3)	2.16 (1.34 – 3.46)	0.001
Recurrent convulsions‡	88 (13.3)	4 (4.5)	0.44 (0.16 – 1.20)	0.088
Dark/Red urine (BWF)	93 (14.0)	12 (12.9)	1.44 (0.79 – 2.59)	0.230
Clinical signs				
General				
Pyrexia (>37.50C)	411 (62.1)	31 (7.5)	0.59 (0.37 – 0.94)	0.026
Hyperpyrexia (≥40.00C)	55 (8.3)	8 (14.5)	1.61 (0.81 – 3.19)	0.814
Hypothermia (<36.00C)	11 (1.7)	1 (9.1)	0.95 (0.14 – 6.28)	0.961
Pallor	338 (51.1)	45 (13.3)	2.40 (1.42 – 4.05)	<0.0001
Clinical jaundice	177 (26.7)	21 (11.9)	1.37 (0.84 – 2.24)	0.214
Respiratory system				
Respiratory distress	554 (83.7)	53 (9.6)	1.06 (0.55 – 2.01)	0.868
Hypoxia (O2 Sats <90%)	49/662 (7.4)	14 (28.6)	3.60 (2.13 – 6.00)	<0.0001

Cardiovascular/hydration				
Severe tachycardia¥	338/629 (53.7)	35/338 (10.4)	1.26 (0.76 – 2.10)	0.366
Temperature gradient	251 (37.9)	29 (11.6)	1.39 (0.87 – 2.23)	0.163
Capillary refill time >2s	135 (20.4)	21 (15.6)	1.95 (1.19 – 3.18)	0.007
Weak pulse	23 (3.5)	3 (13.0)	1.39 (0.47 – 4.10)	0.557
Shock	411 (62.1)	31 (7.5)	0.59 (0.37 – 0.94)	0.026
Sunken eyes	66/634(10.4)	8/66 (12.1)	1.29 (0.65 – 2.61)	0.467
Abdominal				
Splenomegaly (> 2cm)	247 (37.3)	31 (12.6)	1.63 (1.02 – 2.59)	0.040
Hepatomegaly (> 2cm)	228 (34.4)	31 (13.6)	1.84 (1.15 – 2.94)	0.009
Neurological				
Impaired consciousness	82 (12.4)	17 (20.7)	2.61 (1.57 – 4.34)	0.0002
Prostration	43 (6.5)	6 (14.0)	1.52 (0.69 – 3.31)	0.305
Coma	52 (7.9)	9 (17.3)	1.96 (1.02 – 3.73)	0.046

Legend:
*Numbers are N with proportions in parentheses, denominator indicated where data are missing, ¥ Age defined (<11m >160 bpm; 12 -59m >140 bpm; > 60m >100 bpm), *P-value from x2, CI = confidence interval, ‡ >2 in 24hours, BWF =blackwater fever. OR= Odds ratio.*

4.4.3. Laboratory characteristics

The laboratory characteristics of severe malaria patients recruited through this study are summarized in Table 4.2. Parasite density was measured in 584/662 (88.2%) children. The geometric mean (95% CI) parasite density was 58,863 parasites/μL (49,734–69,668). Only a small proportion of patients had low (<500) parasite density: 6/585 (1.0%). Conversely very high parasite density (>50,000) was common 270/585 (46.7%). To my surprise, there was a higher geometric mean (95% CI) of the parasite density among survivors compared to deaths [61,150 (95% CI 51,133– 73,130.) v 41,379 (95%CI 25,061 – 68,323)] though the difference was not statistically significant ($P<0.076$).

The overall median Hb (g/dL) (IQR) was 7.8 (4.9; 10.1) with significantly higher median Hb among survivors compared to deaths [8.0 (5.1; 10.1) v 4.8 (3.3; 9.7); $P<0.001$]. Severe anaemia was an important factor in mortality where it was among 33/63 (52.4%) of the deaths ($P<0.001$). The overall median lactate (IQR) was 2.2 (1.7; 4.0) but was significantly higher (5.6; 2.6,9.4) among the deaths, in whom 34 (54.0%) showed severe lactataemia ($P<0.001$). Hypoglycaemia (<2.2mmol/L) was infrequent; only 21/577 (3.6%) of the children were hypoglycaemic but when present it was associated with a high mortality (6/50; 12.0%; $P<0.001$).

Table 4.2. Laboratory parameters and their association with mortality

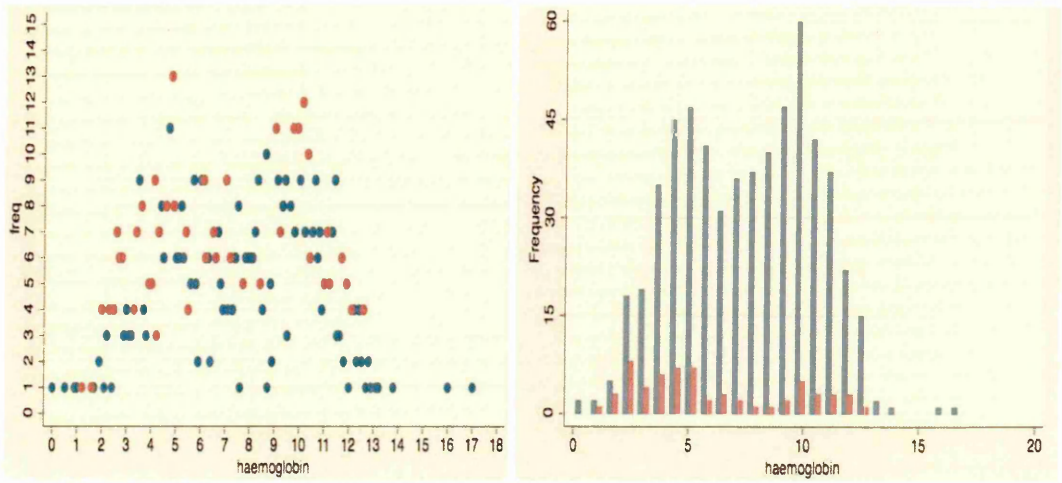
Laboratory parameter	Overall, N (%)	Survivors (%)	Deaths (%)	OR (95% CI)	*P-value*
Number	662 (100.0)	599 (90.5)	63 (9.5)	-	-
Parasitaemia					
Geometric mean (95% CI) *	58 x10^3 (49 - 69 x10^3)	61X10^3 (51 - 73 x10^3)	41 x10^3 (25 - 68 x10^3)	-	-
Hyperparasitaemia#	365/585 (62.4)	331/528 (62.7)	34/57 (59.7)	0.88 (0.51 – 1.53)	0.653
Biochemistry					
Lactate (mmol/L), median (IQR)	2.2 (1.7, 4.0)	2.1 (1.7, 3.6)	5.6 (2.6, 9.4)	-	<0.001
Severe lactataemia (>5mmol/L)	134/660 (20.3)	100/597 (16.7)	34 (54.0)	5.83 (3.40 – 9.9)	<0.001
Blood sugar, median (IQR)	6.8 (5.5, 8.4)	6.8 (5.5, 8.4)	7.5 (5.2, 10.1)	-	<0.001
Hypoglycaemia (<2.2 mmol/L)	21/577 (3.6)	15/527 (2.9)	6/50 (12.0)	4.65 (1.78 – 12.3))	0.001
Haematology					
Haemoglobin, median (IQR)	7.8 (4.9, 10.1)	8.0 (5.1, 10.1)	4.8 (3.3, 9.7)	-	<0.001
Severe anaemia (Hb <5g/dL)	169 (25.5)	136 (22.7)	33 (52.4)	3.74 (2.21 – 6.34)	<0.001
Leucocytes, median (IQR)	11.0 (7.5, 15.7)	10.8 (7.4, 15.5)	12.1 (8.9, 22.7)	-	0.007
Leucocytosis¥	176/632 (27.9)	152/571 (26.6)	24/61 (39.3)	1.79 (1.04 – 3.07)	0.035
Platelets	179 (98.5, 308)	181 (102, 313)	164 (80, 265)	-	0.049
Thrombocytopaenia+	263/632 (41.6)	237/571 (41.5)	26/61 (42.6)	1.05 (0.62 – 1.78)	0.866
Severe thrombocytopaenia++	46/632 (7.3)	39/571 (6.8)	7/61 (11.5)	1.77 (0.77 – 4.07)	0.184

Legend: *Numbers are N with proportions in parentheses, denominator indicated where data are missing, * n=585, CI = confidence interval, IQR= interquartile range, All P-values are from chi-square (x^2). # Malaria parasite count > 250,000 parasites/ µL, ¥White blood cell count >11x10^3 cells/mm3, +Platelet count < 150,000, ++Platelet count of <50,000. OR= Odds ratio*

4.4.4. Severe malarial anaemia (SMA)

Severe malarial anaemia (SMA) (Hb <5g/dL) was present in 169 (25.5%). A majority 152 (90%) were <5 years of age. In this sub-group splenomegaly was found in 101 (59.8%); *P*<0.001. Hyperparasitaemia (>250,000/μl) was in 96 (56.8%); no more common than in the rest of children without severe anaemia. Hyperlactataemia was present in 68/167 (40.7%) children with severe anaemia; *P*<0.001), in whom the clinical features of metabolic acidosis (respiratory distress) were present in 61/68 (89.7%). The case fatality rate (CFR) in SMA was 33/169 (19.5%). A high proportion of the overall deaths 33/63 (52.4%) had severe anaemia compared to the rest of the deaths without anaemia (*P*<0.001). Profound anaemia (Hb <4g/dL) was found in 98/662 (14.8%) and was associated with high CRF 20/98 (20.4%), *P*<0.001. All children (169) with Hb <5g/dL had a blood transfusion according to the current guidelines. Mortality in the transfused group was high 33/169 (19.5%) with profound anaemia (Hb <4g/dL) accounting for 20/33 (60.6%) of the deaths. Generally, mortality was common in patients with a lower Hb (Figure 4.1).

Figure 4.1. Scatter diagram and histogram of haemoglobin with survival



Legend: *Left is the scatter diagram and right is the histogram of haemoglobin levels with survival in childhood severe malaria. Blue colour is for survivors while red colour is for deaths.*

4.4.5. Respiration and airway

Although cough is not through to be a cardinal feature of severe malaria, 130 (19.6%) presented with this symptom, with a high proportion (117; 90.0%) of these being <5 years. Surprisingly, cough was not more frequently associated with respiratory distress. Children with both cough and respiratory distress were fewer compared than those with respiratory distress without cough 94/554 (17.0%). Respiratory distress (defined as increased work of breathing manifested as difficult or deep acidotic breathing) was present in 554 (83.7%) with a majority (513; 92.6%) being <5 years of age and CFR of 53/554 (9.6%). Moreover, among those with high levels of lactate (>5mmols/L), respiratory distress was present in 123/134 (91.8%); P=0.005 with a high case fatality rate of 30/123 (24.4%) compared to children with respiratory distress without hyperlactataemia, P<0.001. Similarly,

among patients with severe anaemia, the prevalence of respiratory distress was high (151; 89.3%; P=0.021) and the CFR was 27/151 (17.9%) compared to those with respiratory distress without anaemia; P<0.001. Figure 4.2 shows the distribution of respiratory distress and overlaps with common complications. Respiratory distress without any other complications occurred in 273/554 children (49.3%) but was associated with a low CFR 9/273 (3.3%). Even though efforts to exclude clinical pneumonia (crackles) were made, it is difficult to confidently exclude pneumonia on a clinical basis alone.

4.4.6. Neurological features

The neurological features surveyed and reported in their order of prevalence both overall and by age are: convulsions 147 (22.2%), recurrent convulsions 88 (13.3%), impaired consciousness 82 (12.4), coma 52 (7.9%) and prostration 43 (6.5%). Fifty-two (7.9%) patients had cerebral malaria defined as coma [unconsciousness = U on AVPU scale or graded on BCS ≤2]. Fifty (96%) were <5 years. Hypoglycaemia among patients with coma at admission was present in 7/52 (13.5%). The case fatality among patients with both cerebral malaria and hypoglycaemia was 2/7 (28.6%). Severe anaemia was found in 11/52 (21.2%) in whom CFR was 4 (36.4%). The overall CFR in cerebral malaria group was high 9 (17.3%), P=0.046. Despite surveying for other neurological features, no patients manifested with abnormal posturing associated with brain involvement including decorticate, celebrate, or opisthotonic postures.

4.4.7. Cardiovascular system and hydration

Clinical shock defined as fever or hypothermia with any one of: CRT>2 seconds, temperature gradient, impaired consciousness, severe tachycardia (age defined), weak peripheral pulse, was common 411 (62.1%), mainly in <5 year olds 377 (91.9%) v 34 (8.3%) *P*=0.460. Severe tachycardia was present in 338/629 (53.7%). Similarly, temperature gradient 251 (37.9%) was a dominant feature in <5 year olds. In addition, prolonged capillary refill time (>2 seconds) a feature of poor outcome in other studies [19, 337] was a common feature 135 (20.4%). On the WHO shock criteria (capillary refill time >3 seconds, weak and fast pulse [254]) was found in only 6/662 (0.9%) children, however, the case fatality rate was very high in this group 2/6 (33.3%).

4.4.8. Abdominal and gastrointestinal system

In addition gastrointestinal complications of vomiting 266/662 (40.2%) and diarrhoea 238/662 (36.0%) were also common, both occurring mainly in children <5 years. Overall, splenomegally (>2cm BCM) was present in 247/622 (37.3%) but was commoner in those with severe anaemia 101/169 (59.8%) than those without *P*<0.001. Comparably, more children with splenomegally were aged <5 years (227; 91.9%) even though this association was not statistically significant *P*=0.570. Hepatomegaly (>2cm BCM) was present amongst 228 (34.4%) and was more common in those with severe anaemia 83/169 (49.1%); *P*<0.001 of whom a majority 72/83 (86.8%) were children <5 years of age *P*=0.0053.

4.5. Severe malaria syndromes and combinations of clinical features

4.5.1. Paired and triple clinical manifestations

Since respiratory distress was present in a majority of patients, that is, 554 (83.7%), it was not surprising that there was a large degree of overlap between respiratory distress and other complications in severe malaria. Table 4.4 shows the respiratory distress-paired-combinations that had a prevalence of ≥10% together with the associated CFRs. In addition, figures 4.2 - 4.5 show overlaps between selected clinical features and their respective mortality among severe malaria patients surveyed at Mbale RRH.

Table 4.3. Summary of significant paired clinical features with respiratory distress

Combination with respiratory distress	N (%)	Case fatality rate (%)	*P-value* *
Severe anaemia (Hb <5 g/dL)	151 (22.8)	27 (17.9)	<0.001
Severe lactataemia (>5 mmol/L)	123 (18.6)	30 (24.4)	<0.001
Impaired consciousness/coma	75 (11.3)	15 (20.0)	0.001
Shock	345 (52.1)	24 (6.9)	0.019
Recurrent convulsions	80 (12.1)	4 (5.0)	0.142

Legend: *Numbers are N with proportions in parentheses, * P-values from* x^2

Figure 4.2. Venn diagram of triad severe anaemia, respiratory distress and impaired consciousness

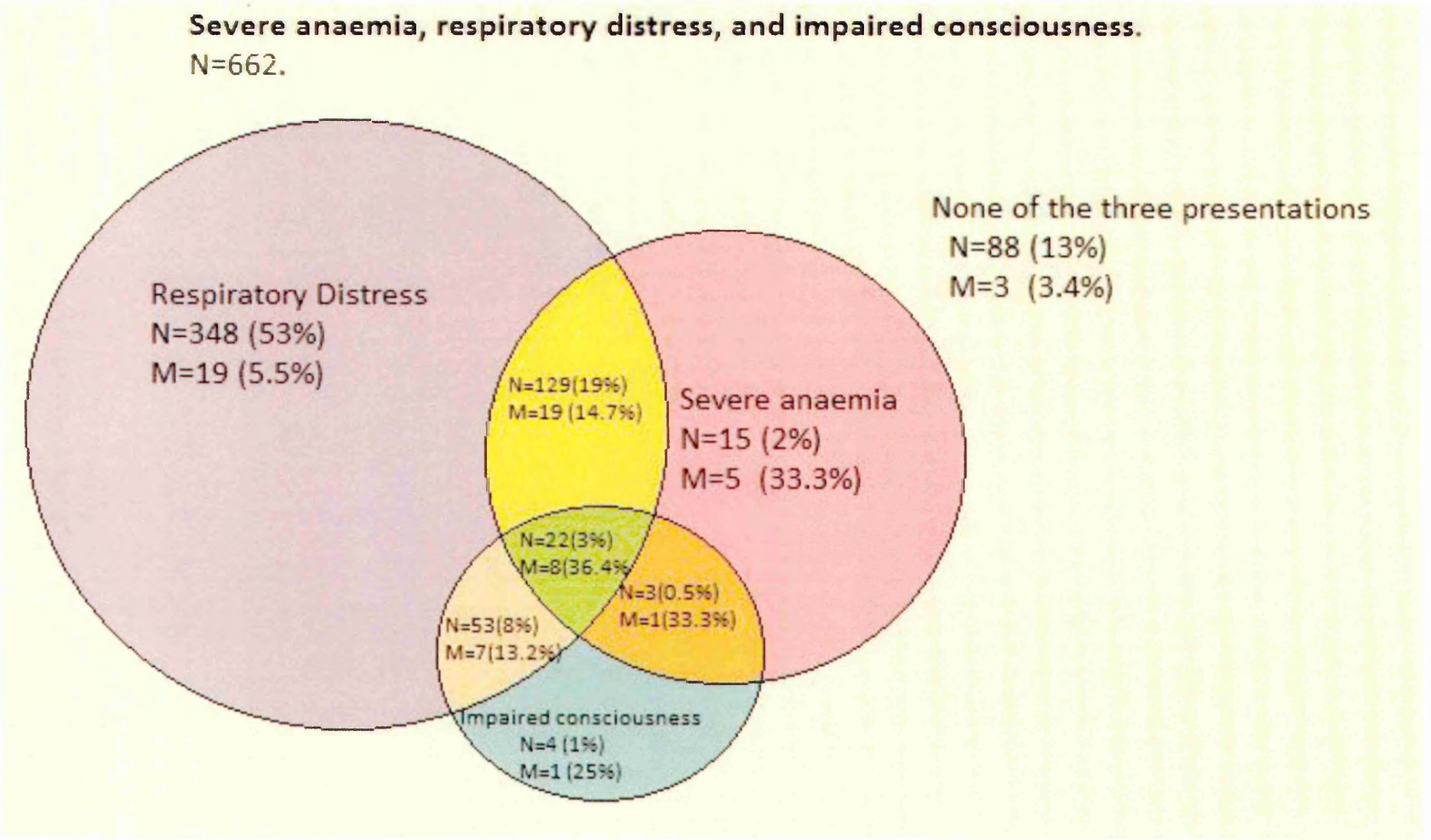


Figure 4.3. Venn diagram of triad severe anaemia, severe lactataemia and hypoglycaemia

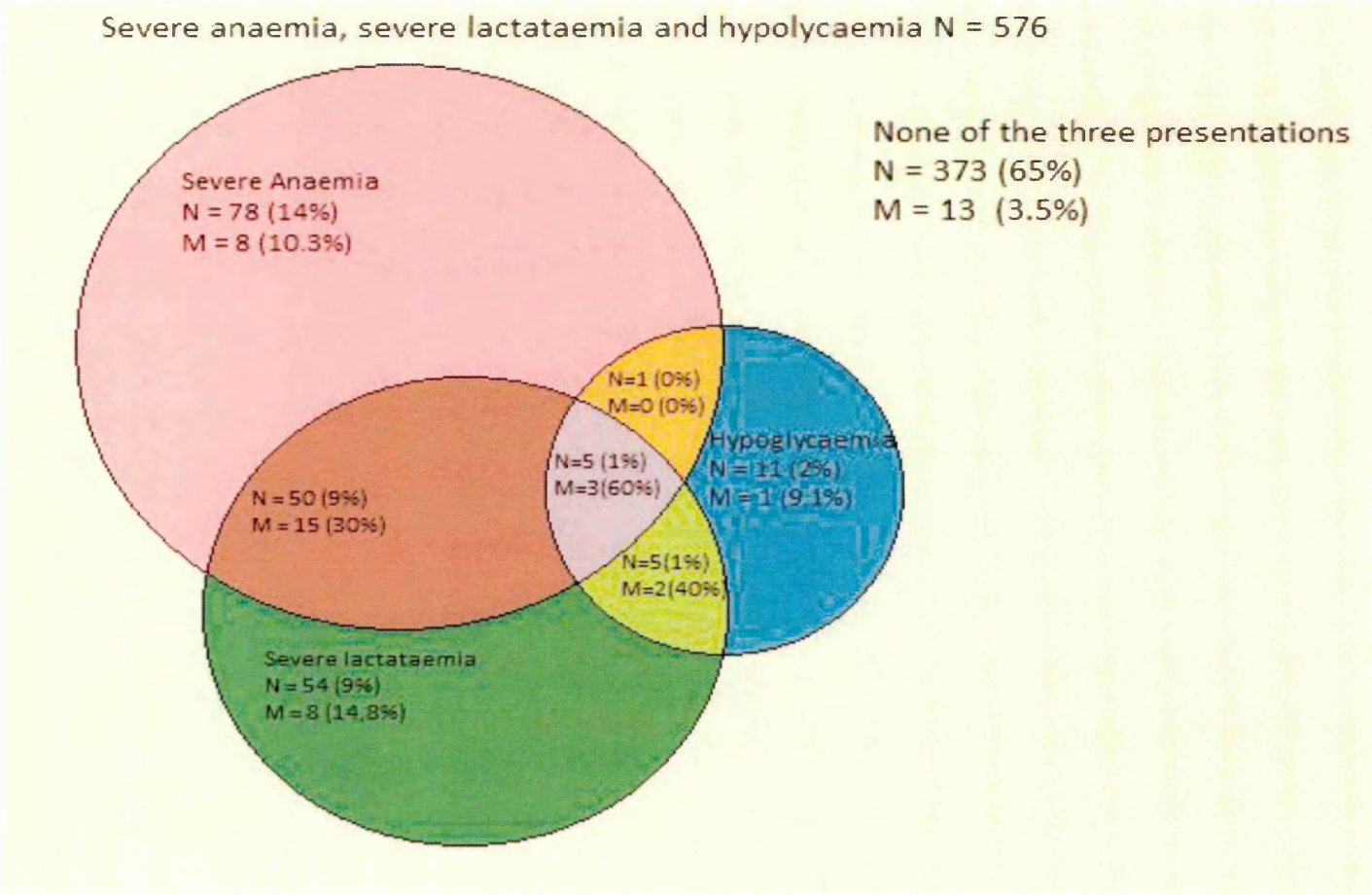


Figure 4.4. Venn diagram of triad severe anaemia, hypoglycaemia and prostration

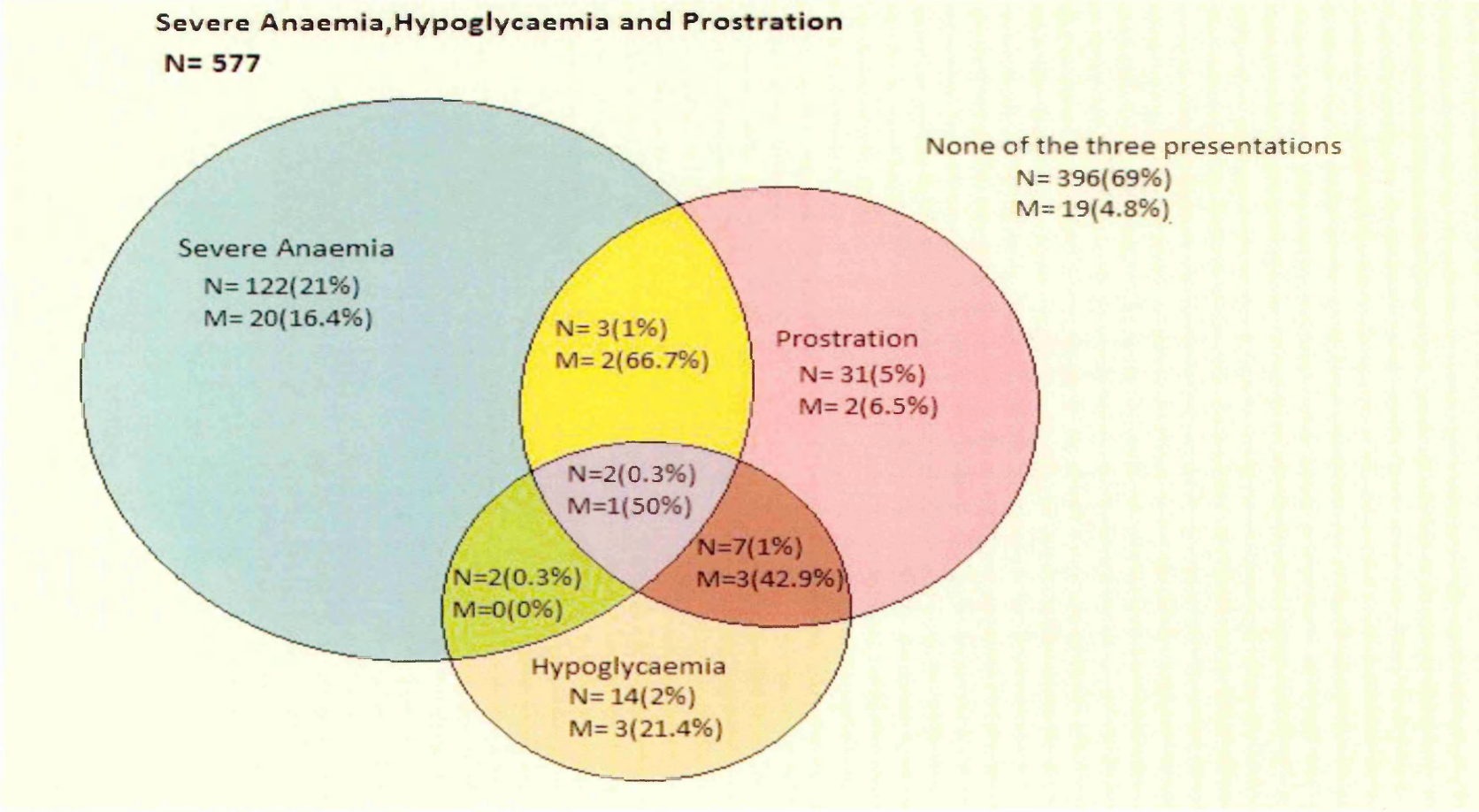
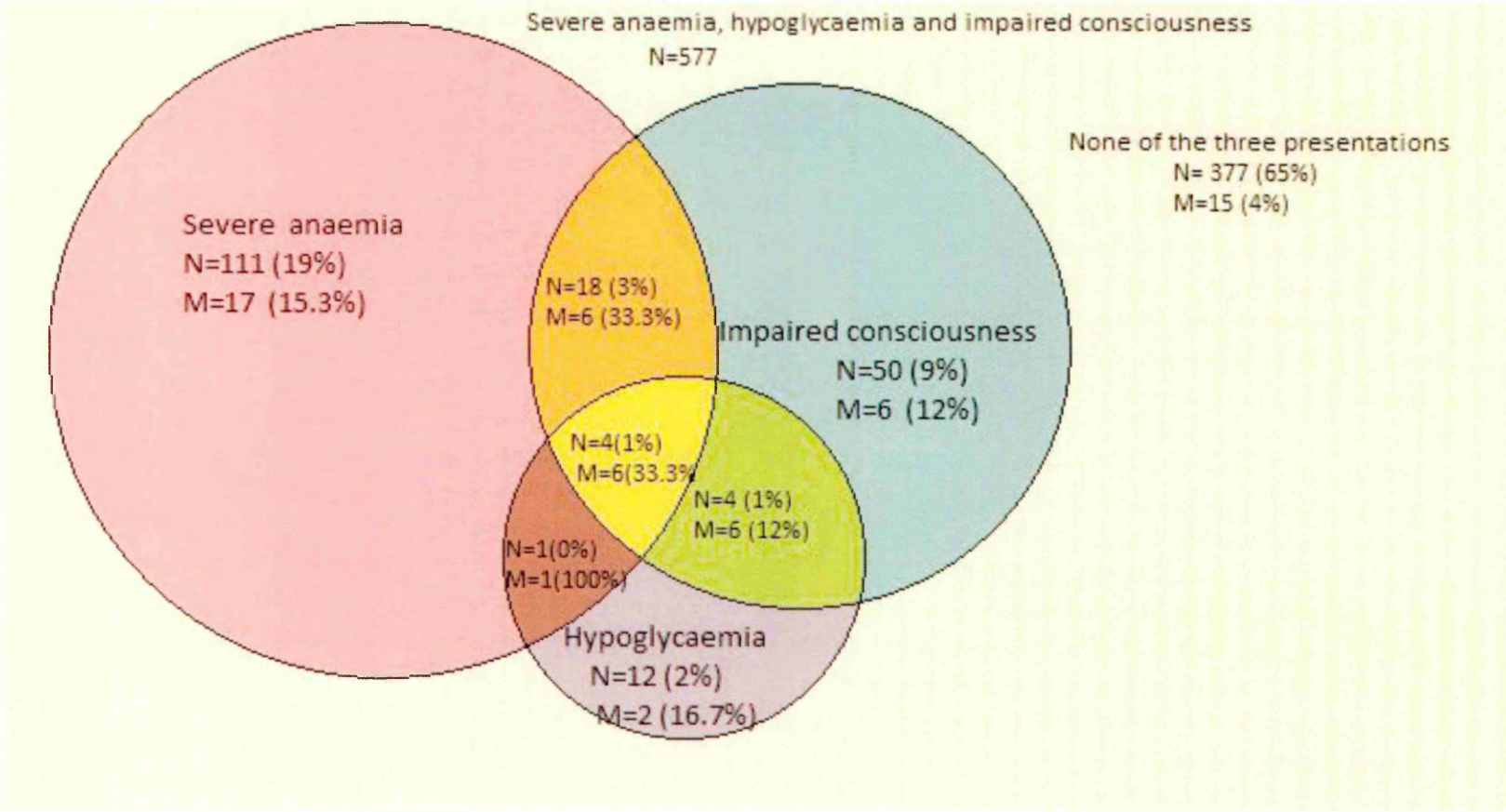


Figure 4.5. Venn diagram of triad severe anaemia, impaired consciousness and hypoglycaemia



4.6. Blackwater fever [Dark Urine Syndrome (DUS)]

DUS accounted for 93 (14.0%) and was associated with a high CFR of 12/93 (12.9%). On ethnicity, most of the patients with DUS were Bantu, 67/93 (72.0%); followed by the Nilohamites 25/93 (26.9%). It was rare in other ethnic groups.

Further, I compared the clinical and laboratory features and their outcomes among children with (n=93) and those without dark urine (n=569) as summarized in Table 4.4. Children with DUS had a significantly higher median age 34 (IQR 17; 52) compared to those without 17 (IQR 9; 29); $P<0.001$, consistent with findings from Nigeria [180] and DRC [179]. However, unlike the series in Nigeria and DRC, it was surprising to note that none of my study participants had clinical features suggestive of acute renal failure. Phenotypically there was no significant difference between in the frequencies in male and female genders $P=0.451$. Clinical jaundice was recorded in a high proportion of patients with malaria related DUS compared to those without [69 (74.2%) v 108 (19.0%); $P<0.001$]. Severe anaemia was more frequent among patients with DUS 40 (43.0%) compared to 129 (22.7%) without DUS; $P<0.001$. In the subsequent two chapters (5 and 6), I explored this syndrome in malaria infection both current and evidence of recent infection.

Table 4.4. Severe malaria: comparison of children presenting with dark urine and without dark urine

Variable	No dark urine	Dark urine	Odd ratio (95%CI)	*P-value**
Number	569 (86.0)	93 (14.0)	-	-
Median Age	17 (9; 29)	34 (17; 52)	-	<0.001
Gender				
Male Sex	325 (57.1)	57 (61.3)	1.19 (0.76 – 1.86)	0.451
Female Sex	244 (42.9)	36 (38.7)		
Ethnicity				
Bantu	491 (86.3)	67 (72.0)	0.41 (0.25 – 0.68)	0.001
Nilohamites	59 (10.4)	25 (26.9)	3.18 (1.87 – 5.41)	<0.001
Luo	10 (1.8)	1 (1.1)	0.61 (0.08 – 4.80)	0.637
Others	9 (1.6)	0 (0.0)		
Clinical criteria				
General				
Clinical jaundice	108 (19.0)	69 (74.2)	12.30 (7.40 – 20.40)	<0.001
Hyperpyrexia	53 (9.3)	2 (2.2)	0.21 (0.0 – 0.81)	<0.020
Neurological				
Impaired consciousness	77 (13.5)	5 (5.4)	0.36 (0.15 – 0.89)	0.027
Recurrent convulsions	82 (14.4)	6 (6.5)	0.41 (0.18 – 0.96)	0.036

Prostration	41 (7.2)	2 (2.2)	0.28 (0.0 – 1.08)	0.067
Coma	50 (8.8)	2 (2.2)	0.23 (0.0 – 0.86)	0.027
Respiratory				
Respiratory distress	490 (86.1)	64 (68.8)	0.36 (0.22 – 0.58)	<0.001
Pulmonary oedema	0 (0.0)	0 (0.0)	-	-
Cardiovascular/hydration				
Shock	363 (63.8)	48 (51.6)	0.61 (0.39 – 0.94)	0.025
Hypotension	4 (0.7)	0 (0.0)	-	0.417
Laboratory				
Mean Hb	8.1 (5.2 – 10.3)	5.7 (4.1 – 8.6)	-	-
Severe anaemia (Hb <5 g/dL)	129 (22.7)	40 (43.0)	2.57 (1.64 – 4.05)	<0.001
Hypoglycaemia (< 2.2 mmol/L)	21 (3.8)	0 (0.0)	-	0.331
Mean lactate (95% CI)	4.24 (2.69 – 4.80)	3.90 (3.30 – 4.59)	-	<0.001
Severe lactataemia (>5 mmol/L)	112 (19.7)	22 (24.2)	1.30 (0.77 – 2.18)	0.323
Outcome				
Mortality	51 (8.9)	12 (12.9)	1.51 (0.77 – 2.94)	0.233

Legend: *Numbers are N with proportions in parentheses, denominator indicated where data are missing, * age in completed months, CI = confidence interval, IQR= interquartile range, *All P-values are from chi-square (x^2)*

4.7. Seasonal trends

This surveillance indicated that admissions due to malaria occurred throughout the year with two typical peak seasons that coincided with rainy seasons (Figure 4.6).

Figure 4.6. Seasonal trends

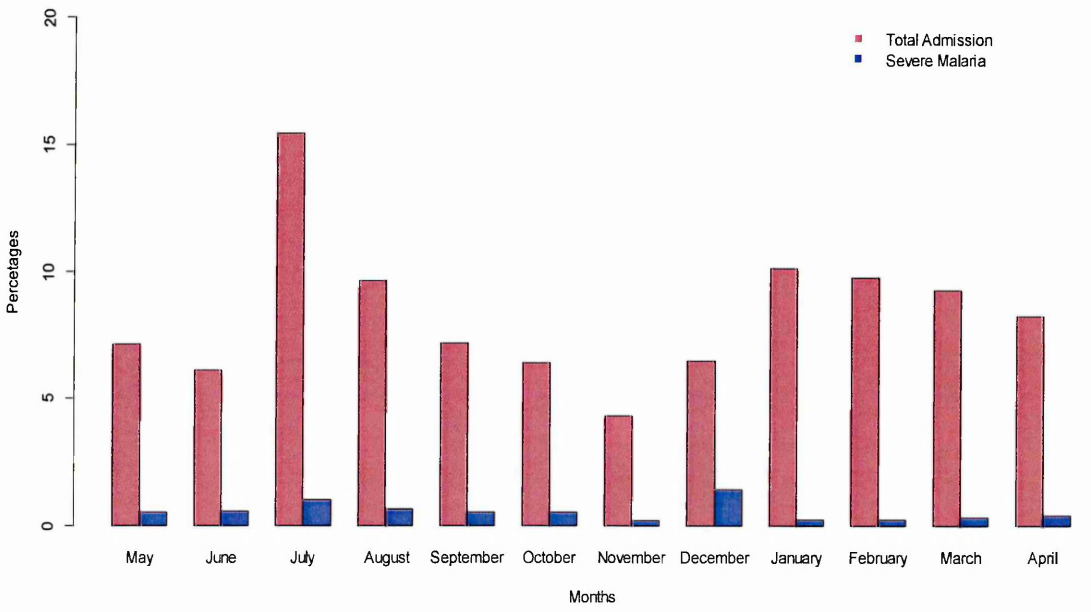


Figure 4.6. Trends of total monthly admissions versus severe malaria admissions May 2011 – April 2012: the total admissions are bars in maroon colour while severe malaria admissions are bars in blue colour. The second seasonal peak of severe malaria set in December 2011 before the peak for total admissions in January-February 2012.

4.8. Multivariate analysis for risk factors for mortality in severe malaria

Data on fifteen clinical and laboratory features of severity were collected and further analysed. The risk of these clinical features for mortality was assessed at two levels. Firstly, at univariate level, the following variables with *P-value* ≤0.05 were identified: pyrexia (*P*=0.026), pallor (*P*<0.001), hypoxia (*P*<0.001), capillary refill time >2seconds (*P*=0.007), splenomegally (*P*=0.040), hepatomegaly (*P*=0.009), coma (*P*=0.046) impaired consciousness (*P*<0.001), severe lactataemia (*P*=<0.001),

hypoglycaemia (P=0.006), severe anaemia (P=0.001) and leucocytosis (P=0.035). In the multivariate model, however, only variables with $P<0.05$ were considered and the following were independently associated with increased risk of mortality: hypoxia (P=0.007), severe anaemia (P=0.028) and severe lactataemia (P=0.001), (Table 4.5).

Table 4.5. Summary of logistic regression analysis for variables predicting mortality in severe malaria among children in Eastern Uganda

Category	Variable	Univariate logistic regression		Multivariate logistic regression	
		Odds ratio (95%CI)	*P-value*	Odds ratio (95%CI)	*P-value+*
General	Pyrexia >37.5	0.59 (0.37 – 0.94)	0.026	0.79 (0.40 – 1.57)	0.504
	Pallor	2.40 (1.42 – 4.05)	<0.001	0.84 (0.34 – 2.12)	0.719
Neurological	Impaired consciousness	2.61 (1.57 – 4.34)	0.002	1.63 (0.51 – 5.21)	0.409
Cardiovascular	CRT >2sec	1.95 (1.19 – 3.18)	0.007	0.82 (0.35 – 1.93)	0.642
Abdominal	Splenomegaly (>2 cm BCM)	1.63 (1.02 – 2.59)	0.040	1.00 (0.46 – 2.18)	0.994
	Hepatomegaly (>2 cm BCM)	1.84 (1.15 – 2.94)	0.009	1.45 (0.70 – 2.98)	0.319
Respiratory	Hypoxia (O_2 Sats <90%)	3.60 (2.13 – 6.00)	<0.0001	3.31 (1.39 – 7.87)	0.007
Laboratory	Severe anaemia (Hb <5 g/dL)	3.74 (2.21 – 6.34)	<0.001	2.60 (1.11 – 6.08)	0.028
	Severe lactataemia (>5 mmol/L)	5.56 (3.25 – 9.49)	<0.001	3.66 (1.72 – 7.80)	0.001
	Hypoglycaemia (<2.2 mmol/L)	4.65 (1.78 – 12.3)	0.006	2.25 (0.63 – 7.98)	0.211
	Leucocytosis(>11.0X10^3cells/mm3)	1.79 (1.04 – 3.07)	0.035	1.03 (0.49 – 2.14)	0.932

Legend: *Numbers are N with proportions in parentheses, denominator indicated where data are missing, * age in completed months, P-value from x^2 except +from logistic regression, CI = confidence interval, CRT = capillary refill time*

4.9. Outcomes

Overall mortality in this severe malaria study group was high 63 (9.5%). The median age of fatalities was lower 18 (IQR 9; 31) compared to survivors 18 (IQR 10; 33); $P<0.001$. Eleven clinical features of disease severity were associated with mortality at univariate analysis. However, three features including severe anaemia (Hb <5.0g/dL), severe lactataemia (>5mmol/L) and hypoxaemia (O_2 Sats <90%) were independent predictors of mortality. Dual combinations had a case fatality rate in the range of (6.9–24%) and triple combinations in the range of (26.3–50.0%).

4.10. Discussion

My study is the first to describe the in-hospital clinical and laboratory features of childhood severe *P. falciparum* malaria using the WHO criteria in Eastern Uganda [1]. Of the 662 children recruited between April 2011 and May 2012, the median age was 18months (IQR 10; 33) and a majority 602 (91.9%) were <5 years of age. The common clinical features were respiratory distress, shock, clinical jaundice, severe anaemia, hyperlactataemia and DUS. The overall case fatality rate was high (63; 9.5%) and increased with multiple combinations of clinical manifestations.

In this study the biggest burden was born by children under 5 years of age most likely because of their poor level of acquired immunity to the disease and background high transmission of malaria [1, 11, 338]. These findings were similar to those reported in previous studies conducted in Kenya [8], The Gambia [216, 339, 340], Malawi [21], and Sudan [341], areas with and/or studies conducted during periods of high malaria transmission. However, the youngest child with

severe malaria in the current study was only 2 months old, probably the youngest reported in the recent literature of patients with severe malaria. In this study complicated and uncomplicated respiratory distress combined accounted for 554 (83.7%), but mortality outcomes were worse in complicated respiratory distress where it probably contributed to the worsening metabolic and major organ functioning; both of which have been cited as mechanisms for poor outcomes in severe childhood illnesses [342]. In my study shock as defined in the FEAST study [159], was common 411 (62.1%); however, the WHO shock criteria (CRT >3 seconds, weak and fast pulse)[254] could only identify few children 6/662 (0.9%) with a high mortality rate 2/6 (33.3%), indicating that this was a very stringent criteria only able to detect shock in late stages of the disease hence too few recognised and often too late to save lives. It was surprising to find a high prevalence 177/662 (26.7%) of clinical jaundice in children with severe malaria. This was contrary to the literature, which in general suggests that jaundice is rare among children with severe malaria. However, it is plausible that its relationship with severe anaemia and DUS, both of which were common in this study population is a pointer to underlying massive haemolysis. This triad of severe anaemia, jaundice and DUS may be of significant relevance unique to this population and is a particular focus of chapters 5 and 6.

Severe malarial anaemia was common. This was not surprising since earlier studies had indicated a link between malaria high transmission and severe anaemia in children, especially those <5 years of age area [8]. My findings also showed variation with series from low transmission areas in which the spectrum includes mainly neurological manifestation in older persons [22, 24, 225].

In a recent meta-analysis of severe malaria conducted among African children from high-endemicity areas, impaired consciousness, respiratory distress, and hypoglycemia were not only the common clinical manifestations but also prognosticators of poor outcomes [18]. However, hypoglycaemia was not frequent 21/577 (3.6%) in my data, though it was associated with a high CFR 5/50 (12.0%), P=0.001. Data on renal failure in childhood malaria has been rarely reported in earlier literature and this could be for a number of reasons. The clinical features associated with ARF may be uncommon in children because of their robust physiology in which progression of renal impairment to fully established renal failure following shock readily reverses following a fluid challenge as earlier described by Maitland *et al* [49]. In addition, clinicians may have a low index of suspicion of this condition, hence under report it. Furthermore, many earlier studies may not have measured renal function in children with severe malaria. Lastly, it could be because of the nature of rapid progression of severe malaria disease in children in which most deaths occur early in the disease; too soon for renal impairment to be identified. However, recently, some studies have reported ARF in severe malaria in African children with blackwater fever (BWF) [179, 180]. My data noted 14% (n=662) of the study subjects had BWF, but unlike the studies in Nigeria and DRC, none of the patients presented with history suggestive of ARF though clinical history alone could not exclude subclinical renal impairment. A large randomised trial, AQUAMAT conducted in 10 sites in 9 countries across Africa showed that renal impairment was among the key independent prognosticators of mortality in childhood severe malaria [61].

As expected, CFR was higher in patients with more than one clinical feature (Figures 4.2 - 4.5). In comparison to the series in Kilifi [8], few 22 (3.0%) patients in my series had a triad of severe anaemia, respiratory distress and impaired consciousness but a higher CFR of 8 (36.4%). Other triads in my study included severe anaemia, impaired consciousness and severe lactataemia 19 (3.0%) patients with CFR of 5 (26.3%) and of severe anaemia, hypoglycaemia and prostration 2 (0.3%) and CFR of 1 (50.0%). The trends in the outcome in these triads were consistent with conclusions made by other researchers that the poor outcomes in severe childhood malaria was worse with multiple combination of clinical features [18, 216].

In this study I identified eleven clinical features that were associated with mortality on univariate analysis, including pyrexia, pallor, impaired consciousness, capillary refill time >2seconds, splenomegally, hepatomegaly, hypoxia, severe anaemia, severe lactataemia, hypoglycaemia and leucocytosis. These are similar to findings from other studies [19, 159]. However, the multivariate logistic regression model identified three features that were independently associated with increased risk of mortality: hypoxia (P=0.007), severe anaemia (P=0.028) and severe lactataemia (P=0.001). Hypoxia has not been frequently reported in other series.

In conclusion the common clinical features of childhood severe malaria among patients admitted to the PACU at Mbale RRH were: respiratory distress, shock, clinical jaundice and severe anaemia. Blackwater fever (dark urine syndrome) and clinical jaundice both of which are rarely reported in other similar studies were

frequent in this study. Mortality was high in this study but higher CFR was among patients with hypoxia 14/49 (28.6%), impaired consciousness 17/82 (20.7%) and coma 9/52 (17.3%). Mortality increased proportionally to the number of complications present in any one patient.

CHAPTER 5: Retrospective Dark Urine Epidemiological Study (REDUES) in East African children

5.0. Abstract

Blackwater fever (BWF) here in referred to as the dark urine syndrome (DUS) remains poorly described among African children. Even less is known in terms of geographical distribution, innate characteristics, immunological, and infectious causation of this syndrome in children. I described DUS in East African (EA) children to understand its distribution geographically, with and without malaria, and complications of severe anaemia, jaundice, renal impairment and their outcomes. A clinical trial - Fluid Expansion As Supportive Therapy (FEAST) in children admitted with severe febrile illness complicated by shock (published in 2011), conducted at 6 sites in EA provided the opportunity for REDUES, a sub-analysis to describe the prevalence, geographical distribution and associated clinical complications of dark urine in the EA countries involved.

Overall numbers included in the trial were 3,170 with a median age of 24 months (IQR 13, 38). In total there were 394 children with DUS, almost all 391/394 (99.2%) patients with DUS were from the Uganda sites with a majority of these children 318 (81.0) presenting to the two centres in Eastern Uganda [Mbale site 180/394 (45.7%) or site specific prevalence of 180/1,240 (14.5%) and Soroti 138/394 (35.0) or site specific prevalence of 138/632 (21.8%)]. The site-specific prevalence of DUS was low 27/234 (11.5%) in Lacor despite having highest prevalence of malaria 171/234 (73.1%) among the Uganda sites. It was even lower 46/747 (6.2%) in

Mulago. A comparison of the current malaria infection (blood slide) between patients with DUS and non-dark urine syndrome (NDUS) shows that more NDUS patients 938/1,484 (63.2%) than DUS 147/300 (49.0%) had current malaria infection ($P<0.0001$). Conversely, evidence of recent infection based on Histidine Rich Protein - 2 (HRP-2) was slightly greater in the DUS 192/246 (78.0%) than 811/1,154 (70.3%), $P=0.014$. To further understand the phenomenon of DUS, we compared the clinical characteristics of patients with DUS with those without here designated as Non-DUS (NDUS), which formed the comparison group. In total the NDUS comprised of 1,552 unmatched children recruited in the two sites (Mbale and Soroti) in Eastern Uganda.

The DUS often presented as combination of jaundice, anaemia and dark urine (JAD), a triad I will be representing as the JAD triad. The clinical characteristics among children with DUS compared to NDUS (comparison group) included 256/318 (80.5%) v 673/1552 (43.4%) with clinical jaundice. Severe anaemia (Hb <5g/dL) accounted for 238/310 (77.0%) in DUS compared to 480/1480 (32.4%) in the NDUS; $P<0.001$. Severe lactataemia (lactate >5mmol/L) was predominant in DUS 204/309 (66.0%) v 560/1450 (38.6%) in the NDUS; $P<0.001$. The risk of renal impairment (BUN >20mmol/L) was marked in DUS group 123/187 (65.8%) v 140/847 (38.6%) in the NDUS; $P<0.001$. However, despite more severity of the clinical features among the patients with DUS, the 48 hour-in-hospital and 28 day mortality was similar in the DUS (12.3% and 12.3%) and NDUS groups (9.9% and 9.9%) respectively. Although there was clinical evidence of acute renal injury, acute renal failure was not reported in the trial and thus less commoner than reported in other studies, suggesting the clinical picture of renal involvement in

DUS is heterogeneous between different paediatric populations in Africa. Whereas, the proportion of children with G6PD deficiency among the DUS was higher 35/224 (15.6%) than NDUS 53/489 (10.8%), the similarity in gender distribution within the DUS does not suggest G6PD deficiency was predominant. In addition, there were no differences in the distribution of proportions of sickle cell anaemia (P=0.247) and thalassaemia (P=0.084) between patient with and those without DUS.

In this REDUES, I found a high prevalence of DUS in children with evidence of recent or concurrent malaria infection in Eastern Uganda, a condition that was rare until the last 5 years according to our clinical records at Mbale RRH. I think that the emergence of DUS in this region may relate to the introduction of artemisinin-based combination therapies, adding to other reports that have linked these agents with delayed-onset haemolysis. Further studies investigating this possibility are required and may prove my hypothesis.

5.1. Introduction

'Black water fever' (BWF), a syndrome characterized by the passage of dark-coloured urine in association with clinical jaundice, is generally considered a rare complication of severe *Plasmodium falciparum* malaria among African children [180]. In the past decades, this phenomenon had been described most frequently as a syndrome associated with malaria affecting non-immune adults often Caucasians in whom the condition was characterized by the acute onset of fever in association with dark urine, jaundice and severe anaemia, commonly complicated by renal failure [167, 169, 175, 183, 194, 239, 343]. Some studies have described the phenomenon of haemoglobinuria and jaundice in which they reported varying prevalence of 6–48% [344, 345] and 11–59% [346-348] of patients with severe malaria respectively. But these were observational studies in adult series involving malaria non-immune patients. A study of severe malaria in Nigerian children reported that haemoglobinuria was present in 19%, and that 6% developed acute renal failure [180]. Furthermore, a recent large (9 countries and 10 sites n=5,246) randomised controlled trial of Artesunate v Quinine (AQUAMAT) in African children <15 years of age with severe malaria indicated that the median blood urea nitrogen (BUN), a maker of acute renal injury was high (>20mg/dL) among the deaths compared to survivors was 23.9 v 14.9; $P<0.001$, and that clinical jaundice was rare but a marker of poor outcomes. Haemoglobinuria was found in 237/5,426 (4.4%) with a case fatality rate of 22/237 (9.3%) [61]. These observations suggest that the spectrum of severe malaria may differ between adults and children in African populations [188, 325, 349, 350]. To date, few published studies of BWF have focused on children [172, 351].

Whereas, BWF has been conventionally used to describe *P. falciparum* complicated by haemoglobinuria with or without renal failure, to my knowledge, a study by O'Donnell *et al,* in Papua New Guinea (PNG) was the first to describe this condition as dark urine syndrome [184]. The authors categorised children with severe malaria into three based on urine colour and its protein content. Group one comprised 22 children with dark urine with a haem-protein, group two had 93 children with normal colour of urine with a haem-protein and group 3 had 236 children with normal urine without haem-protein. Within the patients in group one (dark urine), 14/22 (63.6%) had myoglobinuria, 9/15 (60.0%) had *schistosomiasis* and all the 22 had haemoglobinuria [184]. Therefore, I adopted the use of the term dark urine syndrome (DUS) in this thesis mainly because dark urine potentially has multiple aetiologies.

In my systematic review of BWF (Chapter 1, Table 1.2), no study compared the frequency of BWF (dark urine syndrome) in severely ill children with and without malaria or described the geographical distribution of this syndrome. In addition, no studies have compared clinical features of patients with dark urine with those without dark urine. I have attempted for the first time to describe these especially in East Africa and Eastern Uganda in particular. The REDUES using the FEAST trial data was the most ideal entry point to initial description of this syndrome because it was a multicentre study, involving six sites in the three EA countries, including those in Eastern Uganda.

In the FEAST Trial (2009 - 2011), DUS was reported as a clinical feature at admission in 394/3,170 (12.4%) patients and clinical jaundice in 1,014/3,170

(32.0%). In order to improve my understanding of DUS and its complications, and further guide in the next scientific investigations of this syndrome, I analysed these data to describe the prevalence, geographical distribution and the associated clinical complications of dark urine across each of the six trials sites in the three EA countries involved. In addition, analyses on risk factors for DUS in the two trial sites in Eastern Uganda where this condition was found to be most prevalent were done. As indicated previously in table 1.3, there are many causes of haemolysis in children. Furthermore, drugs have been known to cause dark urine. We were, however, able to investigate the common inherited red blood cell conditions including G6PD, sickle cell disease and thalassaemia in patients with and without dark urine.

5.2. Materials and Methods

The detailed methods and outcome of the FEAST study have been described and published previously [241, 242, 319, 342, 352]. In summary, this was a pragmatic trial in which children with severe acute febrile illness associated with altered consciousness (prostration or coma) and/or respiratory distress (increased work of breathing) plus clinical evidence of impaired perfusion (capillary refill time (CRT) >2seconds or lower limb temperature gradient or weak radial pulse volume or severe tachycardia, were recruited. Children aged 2 to 143 completed months and fulfilling these criteria were randomized to either stratum A or B. They were randomised to stratum A if children had impaired perfusion without evidence of severe hypotension (age categorised) in which they received 20 - 40mL/Kg body weight of 5% Albumin (Albumin-bolus group), 0.9% Normal saline (saline-bolus group) or No-bolus (Control group). Stratum B comprised of children with severe

hypotension and were randomised to the bolus group 40 - 60mL/Kg body weight of either 5% Albumin or 0.9% Normal saline. Minimal exclusion criteria was observed, including: clinical evidence of severe malnutrition in which the use of intravenous fluids are highly contentious, gastroenteritis for which albumin randomization was not appropriate and non-medical conditions including trauma and burns. FEAST study was conducted in six sites in three East African countries: Kilifi, in Kenya; Mbale, Mulago (in Kampala), Lacor and Soroti in Uganda, and Teule in Tanzania. In total 3,170 children were recruited to the trial [319].

5.3. Baseline and follow up data collection in the FEAST Trial

A structured clinical case report form was completed. Admission blood samples were analysed for blood gases and electrolytes, haemoglobin, glucose, lactate, HIV antibody status and *Plasmodium falciparum* [159].

5.4. Dark urine in the FEAST trial participants

Across the six sites we described the demographic characteristics and clinical spectrum of the FEAST trial participants including the prevalence of dark urine (noted as a history of haemoglobinuria in the current illness in the case report form) and clinically evident jaundice. More detailed exploration of the clinical and laboratory data were restricted to the trial participants from Eastern Uganda (Mbale and Soroti sites) among which the highest prevalence of dark urine was seen. The DUS case definition was similar to those in case studies and other descriptive studies on children with BWF [172, 183, 185, 194]. We compared these to participants who gave no clinical history of passing dark urine [non-dark urine syndrome (NDUS)] at these sites.

5.5. Data analysis

Statistical analyses were performed using STATA version 11.0 (STATA Corporation, College Station, TX). Features of clinical severity at recruitment among FEAST trial participants were analysed and presented by site. Continuous variables were summarized using medians with interquartile range (IQR) or means with 95% confidence intervals (CI) as appropriate. Variable analyses have been described in detail in Chapter 2 section 2.8. Factors associated with *P-values* of <0.05 were considered statistically significant.

5.6. Aims and Objectives

In this chapter, I aimed at the following:

1. To describe the prevalence of dark urine syndrome (DUS) in children admitted to hospital with severe febrile illness in 6 centres in East Africa.
2. To compare the clinical features in children presenting with DUS with those without DUS to get a better understanding of:
    a. The clinical spectrum and complications of dark urine syndrome,
    b. To identify potential factors if any, which might have association with the development of dark urine syndrome,
    c. Describe the outcome of dark urine syndrome,
    d. To generate a set of hypotheses with regards to aetiology, complications and outcome that could be explored in future prospective studies.

5.7. Results

5.7.1. Demographic and clinical data across sites

The distribution of clinical severity features, demographic characteristics and mortality across all six sites are summarized in Table 5.1. The median age of FEAST study participants overall was 24months (IQR 13; 38) and was similar across study sites. The proportion of males varied from 47.0% in Teule, Tanzania to 56.0% in Mbale, Eastern Uganda. The overall prevalence of malaria infection was (57.0%), but by-site; it was highest in Lacor, Northern Uganda (73.0%) and lowest in Kilifi, Kenya (41.0%) and ranged between 50.0 - 57.0% at all other sites. Of the 3,170 children recruited to the FEAST trial 2,623 (83.0%) were recruited at three sites in Uganda: Mulago, Mbale and Soroti (Table 5.1).

Table 5.1 Summary of the major clinical and laboratory features of severity within the FEAST study site.

Site	Mbale	Soroti	Lacor	Kampala	Kilifi	Teule
Country	Uganda				Kenya	Tanzania
Demographic features						
Endemicity (malaria)	Holo-endemic	Holo-endemic	Hyper-endemic	Hyper-endemic	Meso-endemic	Meso-endemic
N (%)	1240 (39.0)	633 (20.0)	234 (7.4)	750 (23.7)	216 (6.8)	97 (3.1)
Sex (M) %	698 (56.3)	340 (53.7)	125 (53.4)	394 (52.5)	102 (47.2)	46 (47.4)
Median age*	25 (14, 39)	23 (14, 36)	27 (16, 36)	21 (12, 39)	26 (11, 46)	19 (12, 39)
Clinical features of severity						
Respiratory distress	1222/1236 (99.0)	495/630 (78.6)	125 (53.4)	514/745 (69.0)	177 (82.0)	77/96 (80.2)
Coma	241/1239 (19.5)	61/631 (9.7)	30 (12.8)	57 (7.6)	57 (26.4)	30 (31.0)
Jaundice	759 (61.2)	170/630 (27.0)	28 (12.0)	55/747 (7.4)	1 (0.5)	1 (1.0)
Haemoglobinuria	180 (14.5)	138/632 (21.8)	27 (11.5)	46/747 (6.2)	1 (0.5)	2 (2.0)

Laboratory indicators of severity						
Malaria blood slide +ve	699 (56.4)	388 (61.3)	171 (73.1)	381 (51.0)	88 (40.7)	51 (52.6)
Severe anaemia&	639 (51.5)	340 (53.7)	96 (41.0)	197/743 (26.5)	34 (16.0)	37 (38.0)
Hypoglycaemia¶	46/1134 (4.1)	19/618 (3.1)	21/223 (9.4)	29/710 (4.1)	9/214 (4.2)	12/95 (12.6)
Lactate >5 g/dl	466/1135 (41.1)	301/627 (48.0)	90/210 (43.0)	222/727 (30.5)	55/214 (25.7)	45/95 (47.4)
BUN >20 mmol/L	131/605 (21.7)	134/432 (31.0)	51/159 (32.1)	89/634 (14.0)	25/189 (13.2)	14/90 (15.6)
Outcomes						
Mortality‡	115 (9.3)	58 (9.2)	38 (16.2)	63 (8.4)	21 (9.7)	20 (20.6)

Legend: *Figures are N with proportions in parentheses; denominators indicated where data are missing; * Completed months; &Hb <5g/dl; ¶ Glucose <2.2 mmols/L; ‡At 48 hours.*

5.7.2. Dark Urine Syndrome in Eastern Uganda

The majority of cases of dark urine 318/394 (81.0%) presented to sites in Eastern Uganda, specifically in Mbale and Soroti, where dark urine was reported in 180/1,240 (14.5%) and 138/680 (21.8%) of cases respectively. Clinically evident jaundice was also more prevalent at these sites, occurring in 759 (61.2%) and 170 (27.0%) of children respectively. Moreover, evidence of renal impairment (renal injury on the RIFLE score) was more common in Northern and Eastern Uganda (22.0 - 32.0%) than in other centres (4.0%-15.6%).

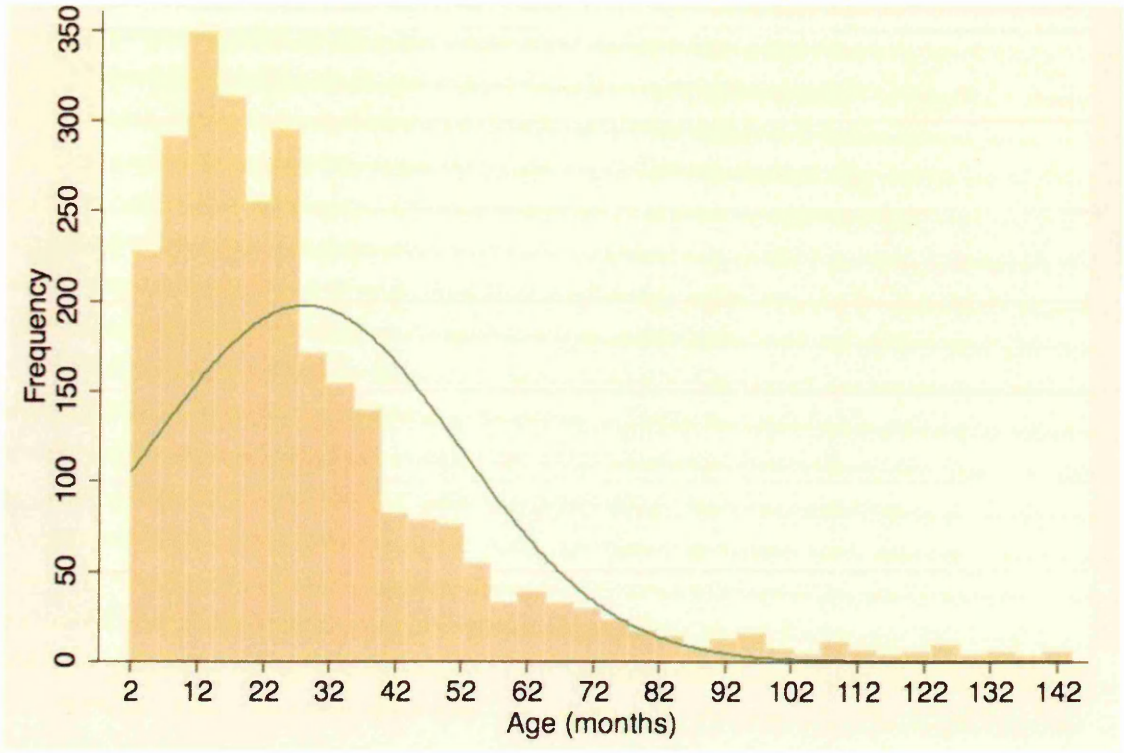
5.8. Features associated with dark urine in Eastern Uganda

Since there was a high prevalence of DUS in Eastern Uganda (Mbale and Soroti sites) I further explored the data from Mbale and Soroti sites to compare the clinical and laboratory features, urine dipstick characteristics and 48-hour and 28-day outcome among children presenting with (n=318) or without a history of dark urine (n=1,552).

5.8.1. Demographic characteristics

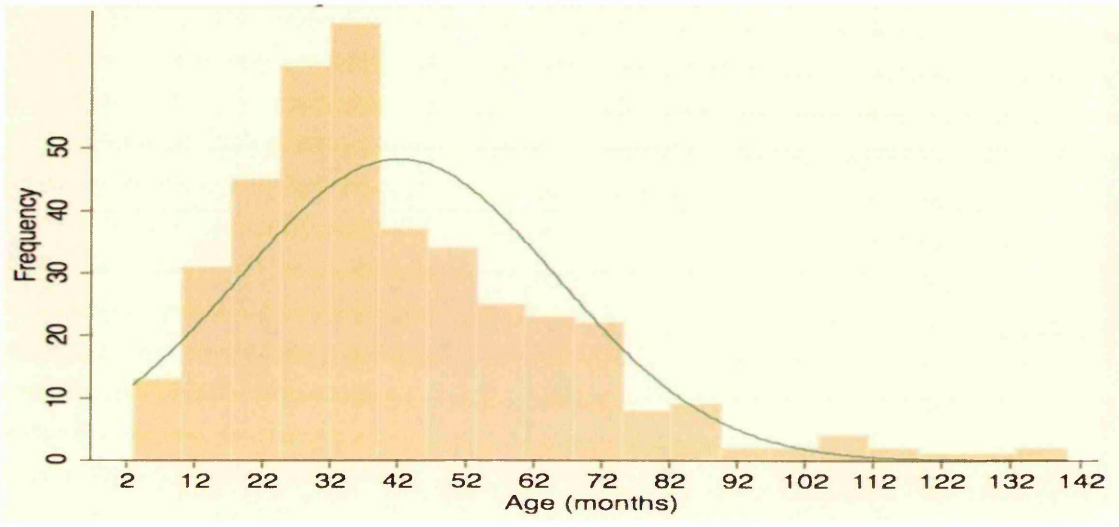
The proportion of males with DUS was only marginally greater 192 (60.4%) than in the NDUS 844 (54.4%) (P=0.050). The median age of DUS patients was significantly older at 36 months (IQR 26, 56) than NDUS at 22 months (IQR 13, 36) (P<0.001) (Figures 5.1 and 5.2).

Figure 5.1. Age Distributions among patients with NDUS in Eastern Uganda



A positively skewed graph indicating a majority of children without DUS were younger with a greater proportion distributed under 40 months

Figure 5.2. Age distributions of among patients with DUS in Eastern Uganda



A positively skewed graph indicating a majority of children without DUS were older than their DUS counterparts with a median age of 36 months.

5.8.2. Clinical characteristics among DUS patients in Eastern Uganda

Children with DUS had a lower overall mean axillary temperature of 37.4°C (95% CI 37.3; 37.6) compared to the NDUS (38.2°C (95% CI 38.0; 38.2). However, this was probably due to the higher frequency of hypothermia (<36°C) in DUS children 35 (11.0%) v 97 (6.3%). Both clinical pallor and jaundice were significantly higher in DUS (83.0% and 80.0%) than in NDUS (33.3% and 43.0)% respectively ($P<0.001$).

Respiratory signs were common overall but more prevalent in NDUS (93.1% v 87.0%); $P<0.001$.

Signs of shock (capillary refilling time >3sec, temperature gradient and weak pulse) [1]; were more common in DUS than NDUS. Despite an older median age than NDUS, the median systolic blood pressure in the DUS group was lower (89 mmHg (IQR 82, 97) supporting the other clinical signs of more severe shock in this group including weak pulse and temperature gradient. Overall, children with DUS had a higher frequency of impaired consciousness (prostration or coma) 83.7% v 70.9%; however, this was largely due to prostration. Neurological manifestations were more prevalent in NDUS than DUS patients including coma (17.7% v 8.5%) and convulsions (13.6% v 4.4%) respectively, Table 5.2.

Table 5.2. Clinical characteristics of patients with a clinical DUS and NDUS in Eastern Uganda (Mbale and Soroti sites)

Characteristics	DUS	NDUS	*P-value**
Demographic features			
Number (%)	318 (17.0)	1552 (83.0)	
Males	192 (60.4)	844 (54.4)	0.050
Age months, Median (IQR)	36 (26, 56)	22 (13, 36)	<0.0001
General Clinical features			
Axillary Temperature °C, Mean (95% CI)	37.4 (37.3; 37.6)	38.2 (38.0; 38.2)	<0.0001
Hypothermia <36.0°C	35 (11.0)	97 (6.3)	<0.0001
Clinical Pallor	263 (83.0)	515/1551 (33.2)	<0.0001
Clinical jaundice	254 (80.0)	673 (43.4)	<0.0001
Respiratory system			
Respiratory distress	275/317 (87.0)	1439/1546 (93.1)	<0.0001
Crackles (pneumonia)	38 (12.0)	359 (23.1)	<0.0001
Circulatory system			
Tachycardia	180 (57.0)	1085/1544 (70.3)	<0.0001
Capillary refill time (=> 3 seconds)	170 (53.5)	348/1550 (22.5)	<0.0001
Temperature gradient	224 (70.4)	968 (62.4)	0.006
Weak pulse	92 (29.0)	361 (23.3)	0.032
Decreased skin turgor	35 (11.0)	127 (8.2)	0.103

Systolic BP (mmHg), Median (IRQ)	89 (82, 97)	92 (85, 101)	0.001
Hypotension	28/312 (9.0)	92/1514 (6.1)	0.120
Nervous system			
Alert	52 (16.4)	451/1549 (29.1)	-
Prostrate	239 (75.2)	824/1549 (53.2)	<0.0001
Coma	27 (8.5)	274/1549 (17.7)	<0.0001
Convulsions at admission	14 (4.4)	209/1549 (13.5)	<0.0001

** P-values reflect x^2 tests for comparisons of proportions and Student's t-tests for comparisons of means.*

5.8.3. Laboratory findings

Malaria parasitaemia was less common in children with DUS compared to NDUS (49.0% v 63.2%) *P*<0.001. The proportion of patients with evidence of recent malaria infection based on HRP-2 was higher 192/246 (78.0%) in DUS patients who had 147/300 (49.0%) current infection based on blood slide as opposed to NDUS in which there was similar proportion of current v evidence of recent malaria infection 938/1,484 (63.2%) v 811/1,154 (70.3%) respectively (Table 5.3).

Children with DUS had a significantly lower baseline Hb 3.7g/dL v 7.1g/dL in NDUS. Overall, 77.0% of DUS patients (n= 310) had severe anaemia (Table 5.3) and thus may have accounted for a much higher frequency of severe acidosis (median lactate 7.9mmols/L v 3.7mmols/L) and elevated anion gap. Evidence of renal impairment, measured by blood urea nitrogen (BUN) was more common in DUS patient with a higher mean BUN (33.0mmols/L v 14.2mmols/L and a nearly four-fold difference in the proportion of DUS patients with high BUN (BUN>20mmols/L) at 65.8% v 16.5%; *P*<0.001. Metabolic and electrolyte perturbations including hypoglycaemia, sodium balance and hypokalaemia were uncommon and did not vary with clinical presentation. Hyperkalaemia (potassium >4.9mmols/L) was common 101/1025 (9.9%) and had significantly higher frequency in children with DUS (17%) v NDUS (8.3%), Table 5.3. The proportion of children with malaria and hyperkalaemia 16/32 (50%) *P*=0.756 were similar to those with malaria, hyperkalaemia and renal impairment 14/28 (50%) *P*=0.960; suggesting that massive intravascular haemolysis may not have been associated with malaria or impaired renal failure alone.

The three commonly inherited red blood cell disorders assessed included sickle cell anaemia, thalassaemia and G6PD. The proportions of each of these were similar in both DUS and NDUS except for thalassaemia homozygous state in which the proportion in DUS was lower 8/216 (3.7%) compared to 81/1,041 (7.8%) in the NDUS. On the other hand the G6PD deficiency was slightly higher in the DUS 15/224 (15.6%) compared to 53/489 (10.8%) (Table 5.3).

Table 5.3. Laboratory parameters among patients with DUS and NDUS in Eastern Uganda (Mbale and Soroti sites)

Parameter	DUS (n=318)	NDUS (n=1552)	*P-value**
Haematology and biochemistry			
Haemoglobin g/dL, Median (IQR)	3.7 (2.9, 4.8)	7.1 (4.3, 9.7)	<0.0001
Severe anaemia (Hb <5g/dL)	238/310 (77.0)	480/1480 (32.4)	<0.0001
Lactate mmol/L, Median (IQR)	7.9 (3.7, 13.2)	3.7 (2.2, 7.7)	<0.0001
Severe Lactataemia (≥5mmol/L)	204/309 (66.0)	560/1450 (38.6)	<0.0001
Anion gap, Mean (95% CI)	20.2 (19.4; 21.0)	17.2 (16.8; 17.6)	<0.0001
Elevated anion gap (>11.0mEq/L)	75/172 (43.6)	153/783 (19.5)	<0.0001
Glucose mmol/L, Median (IQR)	7.9 (5.9, 10.2)	7.0 (5.6, 8.8)	0.0011
Hypoglycaemia (<3mmol/L)	17/303 (5.6)	82/1446 (5.6)	0.967
Sodium, Mean (95% CI)	135.0 (134.3; 135.8)	133.6 (133.2; 133.9)	0.0007
Hyponatraemia (<138mmol/L)	4/190 (2.1)	39/853 (4.6)	0.08
Hypernatraemia (>146mmol/L)	2/190 (1.0)	22/853 (2.6)	0.08
Potassium, Mean (95% CI)	4.7 (4.5; 4.8)	4.2 (4.1; 4.3)	<0.0001
Hypokalaemia (<3.5mmol/L)	0/189 (0.0)	12/836 (1.4)	<0.0001
Hyperkalaemia (≥5.0mmol/L)	32/189 (17.0)	69/836 (8.3)	<0.0001
BUN mmol/L, Mean (95% CI)	33.0 (29.1; 36.7)	14.2 (13.2; 15.1)	<0.0001
High BUN (>20mmol/L)	123/187 (65.8)	140/847 (16.5)	<0.0001

Infection			
Malaria blood slide positive	147/300 (49.0)	938/1484 (63.2)	<0.0001
HRP-2 positive	192/246 (78.0)	811/1154 (70.3)	0.014
Geometric mean HRP2 ng/ml [95% CI]	200 (149, 269)	613 (541, 695)	<0.0001
HIV Antibody positive	4 (1.3)	37/1551(2.4)	0.022
Inherited Red Cell Disorders			
HbS- n (%)			
AA (normal)	199/218 (91.3)	907/1039 (87.3)	
AS (sickle Cell trait)	10/218 (4.6)	64/1039 (6.2)	
SS (sickle cell anaemia)	9/218 (4.1)	68/1039 (6.5)	0.247
Alpha- thalassaemia - n (%)			
Normal	126/216 (58.3)	558/1041 (53.6)	
Heterozygote	82/216 (38.0)	402/1041 (38.6)	
Homozygote	8/216 (3.7)	81/1041 (7.8)	0.084
G6PD deficiency - n (%)			
Normal	173/224 (77.2)	418/489 (85.5)	
Deficient	15/224 (15.6)	53/489 (10.8)	

** P-values reflect x^2 tests for comparisons of proportions and Student's t-tests for comparisons of means.*

5.8.4. Complications of dark urine associated with severe anaemia, jaundice and renal impairment.

It was interesting to note that there were differences in the distribution of clinical jaundice, renal impairment and severe anaemia between the NDUS and DUS patients. Taken alone each of the features were more prevalent in DUS than NDUS. Furthermore, complications as indicated by the overlaps between these features were more frequent in DUS. Surprisingly, however, the case fatality rate among patients who had all the three features was higher in the NDUS 13/55 (23.6%) compared to 8/84 (9.5%). The overlap between clinical jaundice with BUN was more than 4 times in the DUS compared to NDUS (17 (9%) v 14 (2%)) respectively. The overlap between severe anaemia and jaundice was similar in the two groups. The overlap in the three clinical features was, however, more pronounced in the DUS 76 (42%) compared to 42 (5%) in the NDUS (Figure 5.3).

Figure 5.3. Venn diagrams on jaundice, severe anaemia and bun for NDUS and DUS

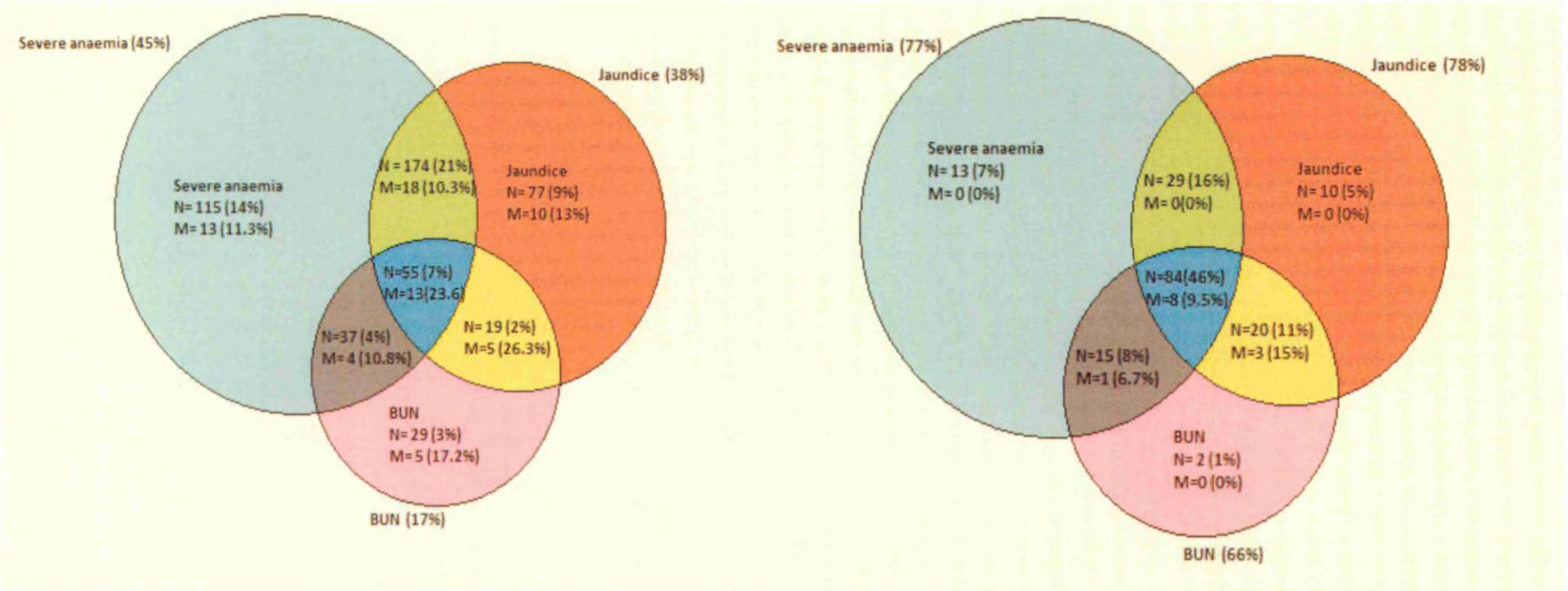


***Figure** 5.3. Showing two Venn diagram each with interactions between clinical jaundice, severe anaemia and BUN. On the right is a Venn diagram for NDUS while on the right is for DUS. Legend: BUN = blood urea nitrogen, Number (%)*

5.8.5. Urine Analysis

A smaller number of urine samples collected at admission were analysed using bedside tests (DUS n=100 and NDUS n=366). The deep cola coloured urine dipsticks were unreadable and the number of these were not recorded. We found important difference in the DUS and NDUS groups. Almost twice (11.2% v 6.4%) the proportion of patients with DUS had non-haemolysed blood (i.e. haematuria); $P<0.001$. As expected a higher proportion (54.3% v 14.7%) of the DUS had haemolysed red blood cells (indicative of haemoglobinuria) but the proportion with urobilinogen (a marker of intravascular haemolysis) was similar. Significant proteinuria (defined as protein detected by multistix test ≥2+, equivalent to or more than 100mg/dL on concentrated or dilute urine) was more prevalent in the DUS category (65.0% v 45.6%). Surprisingly, we noted that Leucocyte esterase (a feature of possible urinary tract infection), was present in 94/463 (20%) with no significant difference between DUS and NDUS (17.0% v 21.2%) ($P=0.5$). This may have been due to misreading resulting from technical difficulties in reading the test strip placed in dark urine, (Table 5.4).

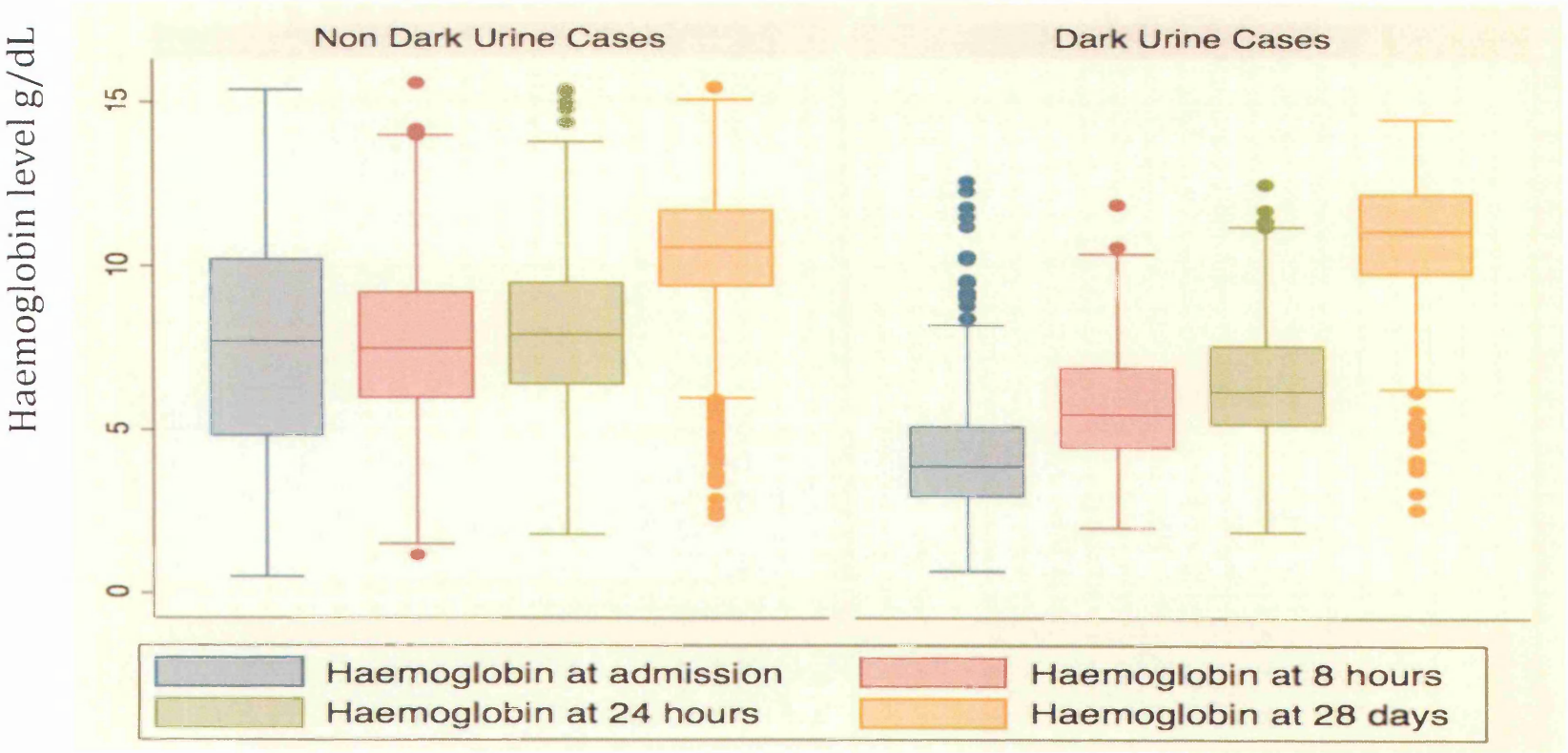
Table 5.4. Urine dipstick results in patients with and without DUS

Parameter	DUS, n/N (%)	NDUS, n/N (%)	*P-value*
Haemolysed RBCs	51/94 (54.3)	46/314 (14.6)	<0.0001
Non-haemolysed RBCs	10/89 (11.2)	19/297 (6.4)	0.3
Both haemolysed and non-haemolysed RBCs	7/89 (7.9)	8/293 (2.7)	0.029
Neither haemolysed nor non haemolysed RBCs	36/89 (40.5)	242/293 (82.6)	<0.0001
High bilirubin	22/100 (22.0)	59/362 (16.3)	0.23
High urobilinogen	8/100 (8.0)	32/358 (9.0)	0.94
Ketones	57/100 (57.0)	144/364 (40)	0.001
Proteinuria	65/100 (65.0)	167/366 (45.6)	0.001
Nitrate	4/100 (4.0)	28/364 (7.7)	0.332
Glycosuria	1/100 (1.0)	5/366 (1.4)	0.551
Aciduria	69/100 (69.0)	271/359 (75.5)	0.63
High specific gravity (SG)	62/100 (62.0)	187/358 (52.2)	0.333
Leucocyte esterase	17/100 (17.0)	77/363 (21.2)	0.50

5.9. Follow-up and outcome

Severe anaemia was more common in children with DUS, being present in 32.4% at 8 hours and 17.6% at 24-hours (12.2% and 6.0% respectively in NDUS). Between the DUS and the NDUS, the mean Hb at both 8hours (5.8g/dL v 7.4g/dL) and at 24 hours (6.5g/dL v 7.7g/dL) remained significantly lower in DUS patients $P<0.001$. However, by Day 28, the mean Hb for the DUS was higher (10.7g/dL vs. 10.2g/dL) than NDUS ($P<0.001$) (Figure 5.4). On Day 28 the NDUS controls had a higher malaria infection rate than DUS (20% v 14.1%). Mortality at 48 hours (10.4% v 8.9%, $P=0.402$) and 28 days (12.3 v 9.9; $P=0.211$) was similar between the two groups, figure 5.4.

Figure 5.4. Comparisons of Haemoglobin distribution between DUS and NDUS from admission to 28 day follow up



Box plots showing haemoglobin levels among the NDUS and DUS on admission (blue), at 8 hours (maroon) at 24 hours (green), and at 28 days (apricot colour). Patients with DUS had a lower Hb level at admission, 8 hours and 24 hours but at 28 days had a slightly higher Hb than the NDUS.

5.10. Discussion

Our study found that in East African children presenting to hospital with severe febrile illness the phenomenon of dark urine syndrome (DUS) was common 394/3170 (12.4%). There was marked geographic variation, with the majority of patients with DUS 318/394 (81.0) coming from Eastern Uganda. Only 147/300 (49.0%) had malaria parasitaemia (or evidence of current infection), the older mean age of DUS children and the lack of male sex predominance would suggest that neither malaria nor glucose-6-phosphate dehydrogenase deficiency as underlying causes of this condition. DUS presented most frequently with complications associated with clinical jaundice in 256/318 (80.5%) and severe anaemia (Hb <5g/dL) in 238/310 (77.0%) indicative of underlying acute severe haemolysis. The urine analysis indicated the presence of both haemolysed (54.0%) and non-haemolysed blood (11.0%) and bilirubin (22.0%) suggestive of both pre-renal and renal contributions in DUS patients but was not exclusive to this group. Clinical jaundice, severe acidosis and evidence of kidney injury were more common in children with DUS. The 48-hour and Day-28 mortality were similar in children with and without DUS.

Haemoglobinuria has long fascinated observers of this phenomenon; however reports from Africa have been limited to case series. Over the last decade there have been two reports detailing the prevalence and spectrum of dark urine in Papua New Guinea (PNG) and West African (WA) children, however both focused upon children with severe malaria. We believe that this is the first study to report this syndrome in both malaria and non-malaria admissions. It is conceivable that the proportion of children with DUS and a positive slide for

malaria was because of potential effect of prior antimalarials and that OptiMAL can only detect parasite-derived lactate dehydrogenase up to 48-hours of parasite clearance.

Overall 394/3,170 (12.4%) of children had DUS proportionately similar to recent data from Nigeria [180]. We noted substantial geographical differences in the DUS frequency, which correlated with malaria endemicity across East Africa. In the sites with high malaria transmission in Eastern Uganda notably; Soroti 21.8% and Mbale 14.5% of trial participants respectively had DUS, compared to only 0.5% at Kilifi; Kenya, the area with the lowest malaria transmission. Thus, malaria exposure is likely to be an important co-factor. In the large multisite clinical trial AQUAMAT involving 5,425 children at 10 centres in 9 countries [353] in Africa representing a wide range of transmission intensities, haemoglobinuria was only reported in 237/5,426 (4.5%) with mortality being similar (9%) to those without haemoglobinuria (10%) [61]. Similarly, clinical evidence of Jaundice was rare, present in only 114 (2%) but was associated with a significantly higher mortality (19% versus 10% in those without jaundice) [61]. Observational unpublished data (Olupot-Olupot and Engoru, 2007 - 2012) indicate that this condition has become more prevalent over recent years, with cases largely in Eastern Uganda with DUS having frequent relapses leading to multiple hospitalizations with similar presentations requiring transfusion and other supportive care. It is difficult to judge whether this is temporal since standardized treatment and disease control guidelines are not in place within the region and whether the incidence of this condition would become less prevalent were these instituted. However, in areas with high malaria transmission, incidence has not changed even with most control

measures in place, in some areas, increases in disease burden have been reported [9, 52].

These data do not support a single haemolytic trigger but instead multifactorial causes including infections (e.g. malaria and others), autoimmune phenomenon, innate characteristics and oxidative agents (especially drugs, herbs and diet). The role of genetics in aetiology of DUS has long been described[354], especially in G6PD [170, 176, 202, 355, 356] and sickle cell anaemia [357]. Our data showed marginal differences in male sex among patients with DUS making G6PD [201, 202, 355, 356, 358] an unlikely major underlying genetic aetiology; it does not however exclude other structural red blood cell membrane defects. In a prospective study in Papua New Guinea [185], of the 351 children studied for the causes of dark urine, 22 (6.3%) had dark urine containing either haemoglobin or myoglobin or both as a result of haemolysis or muscle cell breakdown in severe malaria but severe anaemia was commoner in patients with normal urine suggesting extravascular haemolytic process. These findings may suggest that our dark urine patients had one or both proteins responsible for dark urine. The evidence implicating malaria in the causal pathway is in keeping with historical reports and recent reports in children [172, 190] and involving recent anti-malarial treatment. First, of all the FEAST trial sites, these were situated in the areas of highest malaria transmission. Second, although *P. falciparum* was less prevalent on malaria microscopy in cases than controls, a higher proportion of cases were positive for HRP-2, a marker of recent malaria infection. Moreover, HRP-2 concentrations were significantly lower among cases than controls, observations that together suggest that many of the case-children had received recent

antimalarial treatment and associated with a delayed onset of haemolysis. Finally, the prevalence of two well-documented malaria-protective traits [359] HbAS and alpha-thalassaemia - was significantly lower in cases than in community controls. The protective association between these traits and BWF also implicates malaria in its aetiology within our study population.

Despite lower rates of recovery from severe anaemia at 48 hours, DUS patients by day 28 had a higher Hb compared to NDUS ($P<0.001$). This may be twofold, firstly, a lower malaria infection rate at day 28 of follow up and secondly, higher transfusion rates as reported in observations (Olupot-Olupot and Engoru, 2007-2012 up) may have resulted in a superior bone marrow recovery.

My study, however, had limitations. Firstly the urine data were incomplete because I did not have data on haemoglobinuria and myoglobinuria, but the basis urinalysis done pointed towards findings by O'Donnell et al in PNG [185]. In addition, detailed investigations of FEAST samples including cytokine profiling and genotyping that would inform this study further were not complete. Furthermore, I had no record of history of drug exposure in these patients and lastly investigations into the mechanism of haemolysis especially antibody-antigen complexes based on coombs test was lacking. These stimulated the prospective study in which some of these would be answered. However, in this chapter I have been able to show that DUS was not exclusive to malaria even though a possibility of recent malaria infection, use of oxidative and haemolytic drugs, or other pathogens were not excluded.

In conclusion, DUS in African children is a complex condition with no single aetiology and/ or pathophysiology. I have been able to demonstrate that this condition is not exclusive to malaria alone as previously thought. Severe malaria due to *Plasmodium falciparum* remains a public health problem in the sub Saharan Africa but DUS is rarely reported in African children. Even few data have shown renal involvement in childhood DUS or severe malaria [61, 180], compared to similar studies in adults [181, 182]. Our evidence indicated specific geographical localization of this condition to Eastern Uganda - which was not witnessed at other sites nor in the multicenter AQUAMAT trial. There is urgent need to define clearly its aetiology, address management especially that there are no current guidelines for treatment, prevention and control.

CHAPTER 6: A Prospective Dark Urine Epidemiological Study (PRODUES) among children presenting to Mbale Regional Referral Hospital in Eastern Uganda

6.0. Abstract

In chapters 1 and 5, I showed that the dark urine syndrome (DUS) is a disease with a poorly described paediatric clinical representation among African children. After localizing high prevalence of DUS to Eastern Uganda (chapter 5), I determined to explore in details the uniqueness of DUS in Eastern Uganda in relationship to existing literature; especially findings by O'Donnell *et al* in PNG in which two proteins (haemoglobin and myoglobin) were found in urine and infections (malaria and *Schistosomiasis*) [185]. In addition I explored the links between this syndrome and commonly used antimalarials and antibiotics. Furthermore, innate risk factors were analysed and reported. Lastly, at community level, I wanted to understand the geographical distribution of DUS in Eastern Uganda.

Through a prospective study, I systematically collected patient phenotypic data including drug exposure, investigated laboratory characteristics including baseline haematology, biochemistry, infectious causes and markers of haemolysis, urinalysis and genetics characteristics. Furthermore, using GPS the locations of cases in the community were mapped in relation to physical features and proximity to health facilities in Eastern Uganda.

Between May 2011 and April 2012, I enlisted 1,087 patients with a history of passing dark urine in the present illness of whom 402 qualified the inclusion

criteria on the basis of reported dark urine colour from whom 268 patients met the refined definition of DUS based on caretaker and physician-witnessed urine colour on HCC ≥5. My analyses were based on the 268 patients and their blood and urine samples on which confirmed case definition was made. A majority 191/268 (71.3%) were children <5 years. Males were 172/268 (64.2%) with male: female ratio of 1.8: 1. The majority of patients 253/268 (94.4%) gave a history of fever. The common clinical findings included pallor 220/268 (82.1%) and clinical jaundice 206/268 (76.9%). On the other hand, laboratory findings indicative of haemolysis included severe anaemia (Hb <5g/dL) in 127/268 (47.4%) and haemoglobinuria in 92/165 (55.8%) and reduced plasma haptoglobin [median (IQR) of 0.07 (0.02 - 0.31)]. Evidence of muscle injury measured by positive myoglobinuria was in 59/165 (36.0%). Current malaria infection based on quality controlled malaria blood slide for *P. falciparum* malaria was low 66/268 (24.6%), but evidence of recent malaria based on HRP-2 was higher 142/268 (53.0%). On innate characteristics, G6PD-deficiency was in 36/268 (13.4%).

The community GPS findings indicated that cases and poor outcomes were frequent in children from the marshy lands in the rural areas.

These findings point to the fact that the aetiology of DUS is multifactorial and not limited to a single aetiology alone as may have been earlier described. It is therefore best described as a syndrome, hence, Dark Urine Syndrome (DUS).

6.1. Introduction

The review of DUS (Chapter 1) indicates that there are gaps in literature on DUS in African children. Chapter 5 revealed that DUS was more prevalent in Eastern

Uganda than any other part in East Africa. It was therefore my conviction that the clinical syndrome associated with dark urine in Eastern Uganda may be of unique significance to the population in the Eastern region of Uganda.

I aimed at providing a comprehensive description of children with DUS, which will incorporate sociodemographic, clinical including use of antimalarial remedies, laboratory including innate characteristics and community based features in an area of intense malaria transmission, which will be of relevance to researchers and clinicians. In particular, unpublished data from the Eastern region of Uganda and the surrounding geographical locations indicate that there is an unusually high incidence of DUS that is characterised by an acute onset of dark urine, jaundice and severe anaemia. In this chapter therefore I investigated the clinical epidemiology of the DUS commonly seen in this region.

6.2. Aims and Objectives

In this chapter, I aimed at the following objectives:

I. To identify all children presenting to the PACU at Mbale RRH with confirmed dark urine syndrome.

II. To describe the clinical features of patients presenting with the dark urine syndrome.

III. To describe the frequency of complications of jaundice, DUS and severe anaemia both individually and as a triad among patients with DUS at Mbale Regional Referral Hospital.

IV. To describe the outcome for factors associated with dark urine in Eastern Uganda.

V. To examine the relationship between haemoglobinuria with the key correlates of severity of malaria among critically sick children at admission.

VI. To describe the common genetic risk factors of dark urine in children.

VII. To describe community distribution of DUS in Eastern Uganda.

6.3. Materials and methods

A detailed description of materials and methods has been covered in chapter 2 sections 2.4 - 2.11, but briefly, this prospective dark urine epidemiological study (PRODUES) was a sub study of the WSS. Only patients with a refined case definition qualified for the PRODUES and therefore my analyses and results were based on the 268 confirmed cases (Chapter 2 section 2.4). Data on sociodemographic and clinical features were manually collected on a customized CRF (Appendix: data collection tools). In addition, specific laboratory analyses and community GPS were also done.

A case-control study to explore the potential risks of developing DUS associated with inherited red cell disorders was done. The cases were 268 with confirmed case definition of dark urine, while the unmatched controls were 496 newborns, making a ratio of cases to controls of 1: 1.85. Blood from cases and controls was genotyped for G6PD deficiency, the mutation conferring sickle cell trait (HbAS) and sickle cell anaemia (HbSS) and □lpha-thalassaemia. The frequency of these

inherited disorders was calculated in both the cases and controls, their Odd's ratios and *P-values* were computed as a measure of association of genes of interest with a likelihood of having dark urine. The results were summarized and reported in a table.

The GPS was used to map the distribution of cases in the community and describe their association with environmental risk factors.

The bedside tests, haematological, parasitological and immunological assays requiring fresh samples were performed at Mbale RRH. In addition, molecular assays were performed using DNA PCR techniques for G6PD, sickle cell anaemia and alpha-thalassaemia.

DUS cases were managed according to the standard of care available at the PACU and follow up was done to assess clinical and laboratory states of convalescent patients. Standardised data analysis methods were applied. All the necessary ethical approvals to conduct the DUS surveys, storage of samples and further analyses abroad were secured.

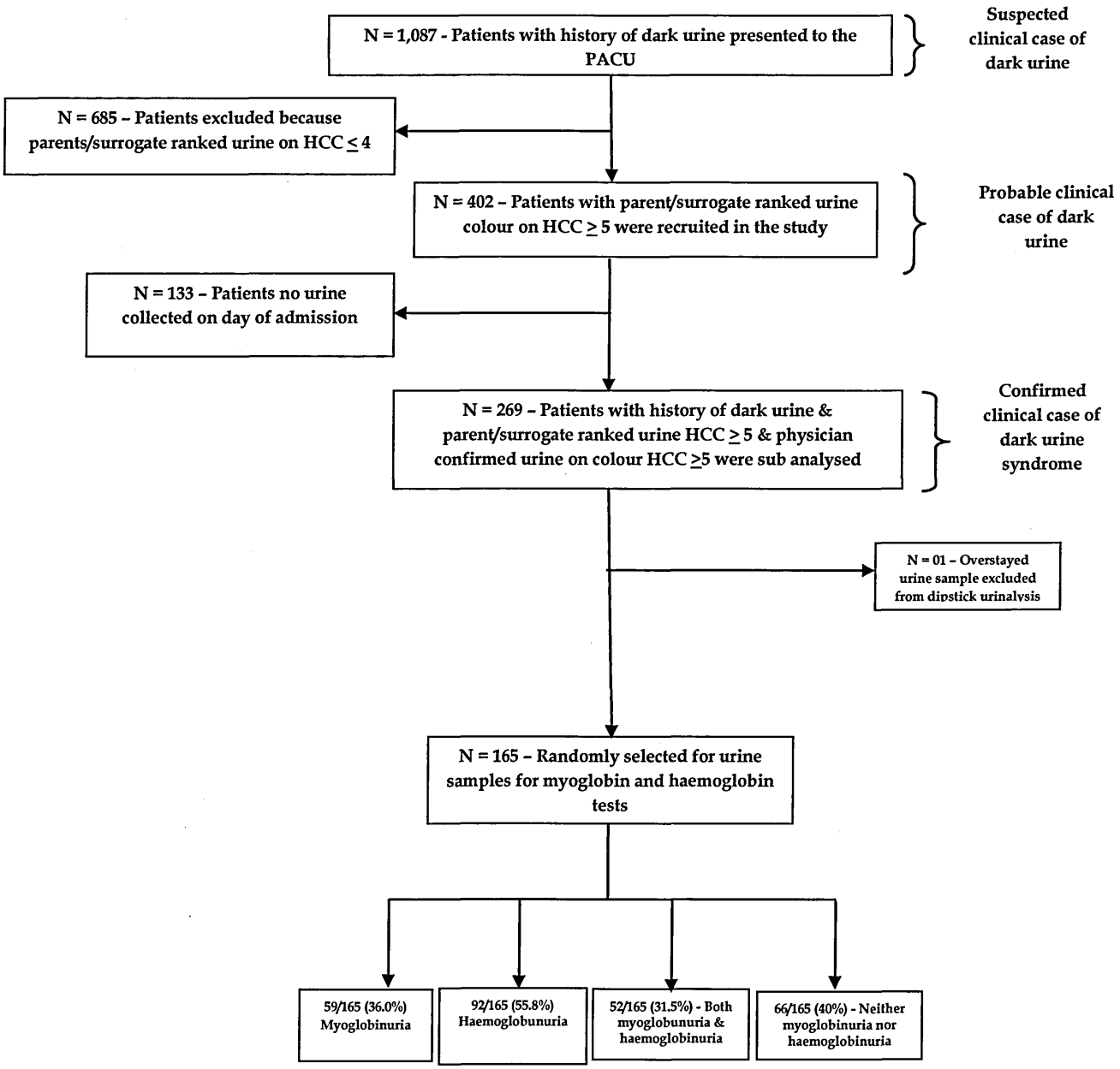
6.4. Results

6.4.1. Definition of the dark urine syndrome

In order to have a clearly defined group of patients on whom I made deductions and conclusions, I classified patients with history of dark urine into three. A suspected case was a child aged 2 -143 completed month who presented with a complaint of passing dark urine in the current illness N=1,087 (100%). From these, a probable case was one whose caretaker showed a patient's urine color

corresponding to Hammersmith urine color chart (HCC) >5 and there were 402 (37.0%) children. Of these, a confirmed case of dark urine was one whose urine colour was witnessed and confirmed by a clinician to correspond to HCC >5; 268/402 (67.0%) patients.

Figure 6.1. Study flow chart for dark urine syndrome in Mbale RRH



6.4.2. Demographic features

Of the 268 confirmed cases, median age 42 months (IQR 26.5, 62.5), the majority 191 (71.3%) were children <5years similar to previous reports on childhood DUS [179, 180]. The proportion of males 64.2% (n=268) was higher than females 35.8% (n=268), but the differences were not statistically significant P=0.660, similar to findings in Nigeria [180] and DRC [179]. On ethnicity the majority were Bantu 187 (69.8%) followed by Nilohamites 77 (28.7%). On the other hand, the Luo, Hamites and others; put together were a minority with less than 3%. This is the largest report describing ethnicity in dark urine syndrome since most published work has been case studies citing Bantu ethnicity [172]. Almost all 249 (93.3%) of the study participants came from rural areas with only 6.7% from urban areas.

6.4.3. Clinical features

Among the 268 confirmed cases, the common complaints included fever 253 (94.4%), cough 189 (70.5%) and vomiting 182 (67.9%), all suggestive of infections. Pyrexia was in 56.0% (n=216), Pallor in 82.2% and clinical jaundice in (76.9%).

One hundred and sixty four (61.2%) children presented with respiratory distress defined as increased work of breathing (either chest indrawing or deep-breathing or both), while pneumonia was present in 30 (11.2%). A substantial number of children presented with features of clinical shock. For instance, 163 (60.8%) patients had severe tachycardia, 86 (32.1%) had a CRT >2 sec and 68 (25.4%) had a temperature gradient. Splenomegaly was found in a high proportion of children (125; 46.6%), (Table 6.1).

Table 6.1. Clinical and demographic characteristics of 268 patients with confirmed dark urine syndrome

Category	Variable	N (%)	Case fatality	OR (95% CI)	*P-value*
Sociodemographic	Patients N (%)	268 (100.0)		-	-
Age group	<5 years	191 (71.3)	25/191 (13)	1.78 (0.72 – 4.41)	0.219
	>5 years	77 (28.7)	6/77 (7.8)	0.56 (0.23 – 1.39)	
Sex	Male	172 (64.2)	21/172 (12.2)	1.19 (0.55 – 2.61)	0.660
	Female	96 (35.8)	10/96 (10.4)	0.84 (0.38 – 1.83)	
Residence	Rural	249 (93.3)	31/249(12.4)	-	
	Urban	18 (6.7)	-	-	-
Ethnicity	Bantu	187 (69.8)	24/187 (12.8)	1.56 (0.66 – 3.68)	0.324
	Hamites	1 (0.4)	-	-	0.717
	Nilohamites	77 (28.7)	7/77 (9.1)	0.69 (0.29 – 1.66)	0.421
	Luo	2 (0.7)	-	-	0.607
	Others	1 (0.4)	-	-	0.717
Clinical history	Fever	253 (94.4)	30/253 (11.8)	-	0.541
	Cough	189 (70.5)	25/189 (13.2)	1.85 (0.75 – 4.58)	0.189
	Vomiting	182 (67.9)	25/182 (13.7)	2.12 (0.86 – 5.23)	0.106
	Diarrhoea	22 (8.2)	3/22 (13.6)	1.23 (0.37 – 4.16)	0.751

	Bloody diarrhoea	24 (9.0)	6/24 (25.0)	2.92 (1.09 – 7.84)	0.031
	Convulsions	20 (7.5)	3/20 (15.0)	1.38 (0.41 – 4.73)	0.618
Clinical features					
General	Pyrexia (>37.50C)	121/216 (56.0)	17/121 (14.0)	1.78 (0.75 – 4.22)	0.199
	Hyperpyrexia (>39.50C)	2/216 (0.9)	2/2 (100.0)	-	<0.001
	Hypothermia (<36.00C)	3/216 (1.4)	1/3 (33.3)	-	0.236
	Pallor	220 (82.1)	30/220 (13.6)	-	0.023
	Clinical jaundice	206 (76.9)	21/206 (10.2)	0.59 (0.26 – 1.31)	0.200
Respiratory	Hypoxia (O2 Sats <90%)	12 (4.5)	4/12 (33.3)	4.24 (1.27 – 14.24)	0.016
	Respiratory distress	164 (61.2)	22/164 (13.4)	1.64 (0.73 – 3.63)	0.235
	Pneumonia (crackles)	30 (11.2)	4/30 (13.3)	1.20 (0.41 – 3.56)	0.748
Neurological	Impaired consciousness	9/261 (3.4)	5/9 (55.6)	11.88 (3.21 – 43.83)	<0.001
	Prostration	8/261 (3.1)	4/8 (50.0)	8.63 (2.23 – 33.48)	<0.001
	Coma	15 (5.6)	6/15 (40.0)	6.08 (2.08 – 17.90)	<0.001
Cardiovascular	Severe tachycardia	163 (60.8)	15/163 (9.2)	0.56 (0.27 – 1.18)	0.131

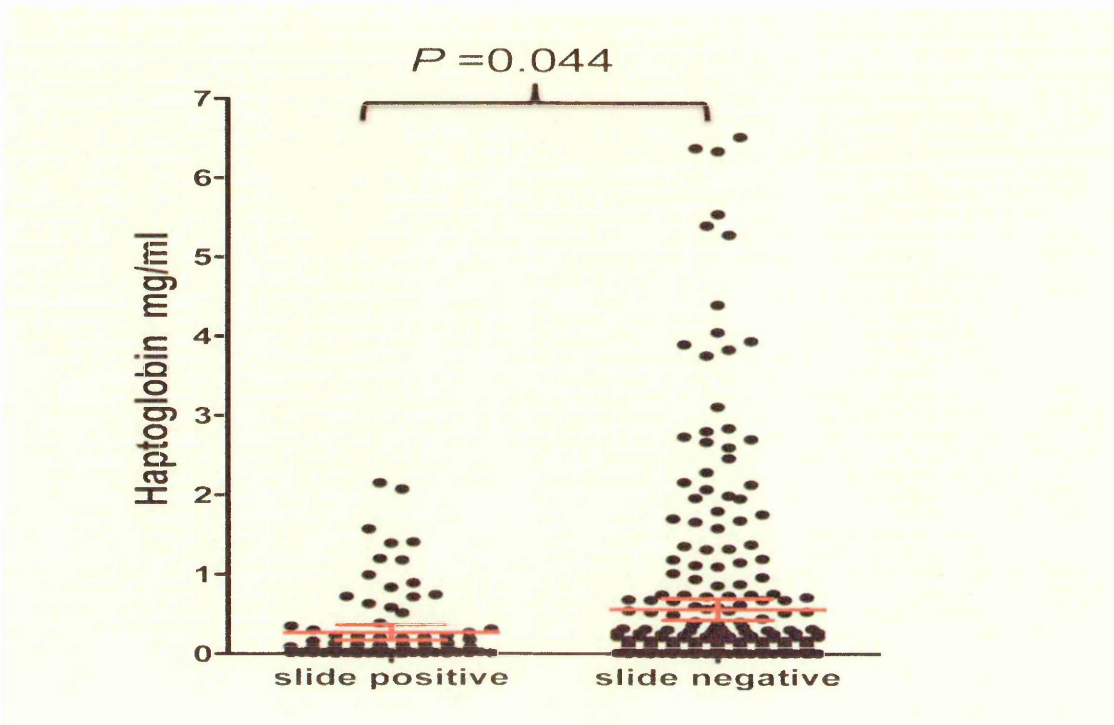
	Temperature gradient	68 (25.4)	10/68 (14.7)	1.47 (0.66 - 3.26)	0.349
	CRT >2s	86 (32.1)	15/86 (17.4)	2.19 (1.04 - 4.62)	0.038
	Weak pulse	14 (5.2)	4/14 (28.6)	3.25 (1.00 - 10.62)	0.049
	Shock	105 (39.2)	15/105 (14.3)	1.53 (0.73 - 3.20)	0.264
Abdominal	Splenomegaly	125 (46.6)	16/125 (12.8)	1.25 (0.59 - 2.62)	0.555
	Hepatomegaly	100 (37.3)	13/100 (13.0)	1.25 (0.59 - 2.63)	0.572

Legend: *Numbers are N with proportions in parentheses, denominator indicated where data are missing, P-value from x^2, CI = confidence interval, OR= Odds ratio*

6.5. Laboratory findings

The prevalence of malaria parasitaemia was surprisingly low in this study population. Only 66 (24.6%) were slide positive for malaria parasites, the proportion being similar among those who survived and those who died (Table 6.2). Nevertheless, a higher proportion of cases were positive for HRP-2 (Histidine Rich Protein-2) 142/268 (53.0%), suggesting that a higher proportion had suffered a recent malaria infection. Haptoglobin concentrations were significantly lower among malaria slide positive than malaria slide negative children, suggesting higher levels of intravascular haemolysis (Figure 6.2).

Figure 6.2. Relationship between malaria infection and plasma haptoglobin levels



Dot plot showing a small portion of patients with DUS had malaria on admission (left) but these patients had lower levels of haptoglobin compared to those without malaria (right).

Hypoglycaemia (<2.2mmol/L) was rare 3.7% and there was no significant difference between survivors and deaths *P*=0.423 (Table 6.2). The median leucocyte level was 10.7 (IQR 7.5, 10.0) with a much higher median 13.1 (IQR 9.3, 28.1) among deaths compared to survivors *P*=0.036. None of the patients had schistosomiasis. They were not tested for trypanosomiasis or filariasis because of logistical reasons. Only one patient had a positive coombs test.

Table 6.2. Laboratory characteristics of 268 children with dark urine syndrome and their association with survival

Category	Parameter	N (%)	Survived	Died	OR (95% CI)	*P-value*
Patients	Number N (%)	268 (100.0)	237 (88.4)	31 (11.6)	-	-
Parasitology	Malaria slide positive	66 (24.6)	59 (24.9)	7 (22.6)	0.88 (0.37–2.10)	0.777
Immunology	HPR2* positive	142 (53.0)	126 (53.2)	16 (51.6)	0.94 (0.45–1.96)	0.871
Biochemistry	Lactate (mmol/L), median (IQR)	2.4 (1.9, 4.3)	2.3 (1.8, 4.0)	2.1 (5.1, 9.0)	-	0.002
	Severe lactataemia (>5mmol/L)	61 (22.7)	45 (19.0)	16 (51.6)	4.55 (2.12–9.78)	<0.001
	Blood sugar, median (IQR)	5.8 (7.4, 8.9)	5.8 (7.3, 8.9)	7.6 (6.3, 8.7)	-	0.724
	Hypoglycaemia (<2.2mmol/L)	10.0 (3.7)	8.0 (3.4)	2 .0 (6.5)	1.91 (0.0–8.41)	0.423
Haematology	Haemoglobin (g/dL), median (IQR)	5.1 (3.9, 8.0)	5.4 (4.0, 8.3)	4.2 (2.9, 5.3)	-	<0.001
	Severe anaemia (Hb <5g/dL)	127 (47.4)	104 (43.9)	23 (74.2)	3.68 (1.61–8.38)	0.001
	Leucocytes, median (IQR)	10.7 (7.5, 10.0)	10.4 (7.4, 15.9)	13.1 (9.3, 28.1)	-	0.036
	Leucocytosis	66/225 (29.3)	54/199 (27.1)	12 (38.7)	2.30 (1.02–5.21)	0.045
	Thrombocytopaenia	82/227 (36.1)	76/201 (37.8)	6/26 (23.1)	0.49 (0.19–1.25)	0.141
	Platelets	190 (119, 281)	188 (116, 267)	234 (155, 326)	-	0.068
	Severe thrombocytopaenia	14/227 (6.2)	14/201 (7.0)	0/26 (0.0)	-	-
	Elliptocytes	32 (11.9)	30 (12.7)	2 (6.5)	0.48 (0.0–1.90)	0.316
	Spherocytes	32 (11.9)	30 (12.7)	2 (6.5)	0.48 (0.0–1.90)	0.316

*Numbers are N with proportions in parentheses, denominator indicated where data are missing, *Histidine-Rich-Protein-II, P-value from x^2, CI = confidence interval. OR= Odds ratio.*

6.5.1. Comparison of laboratory characteristics in blood samples collected from children with DUS at presentation and during convalescence.

6.5.1.1. Plasma haemoglobin

There was evidence of hemolysis during the acute phase of an episode of DUS. A comparison of plasma haemoglobin between acute cases and convalescent cases indicated high levels of plasma haemoglobin in the acute group of patients; $P<0.0001$ (Figure 6.3).

Figure 6.3. Levels of plasma haemoglobin between acute and convalescent samples

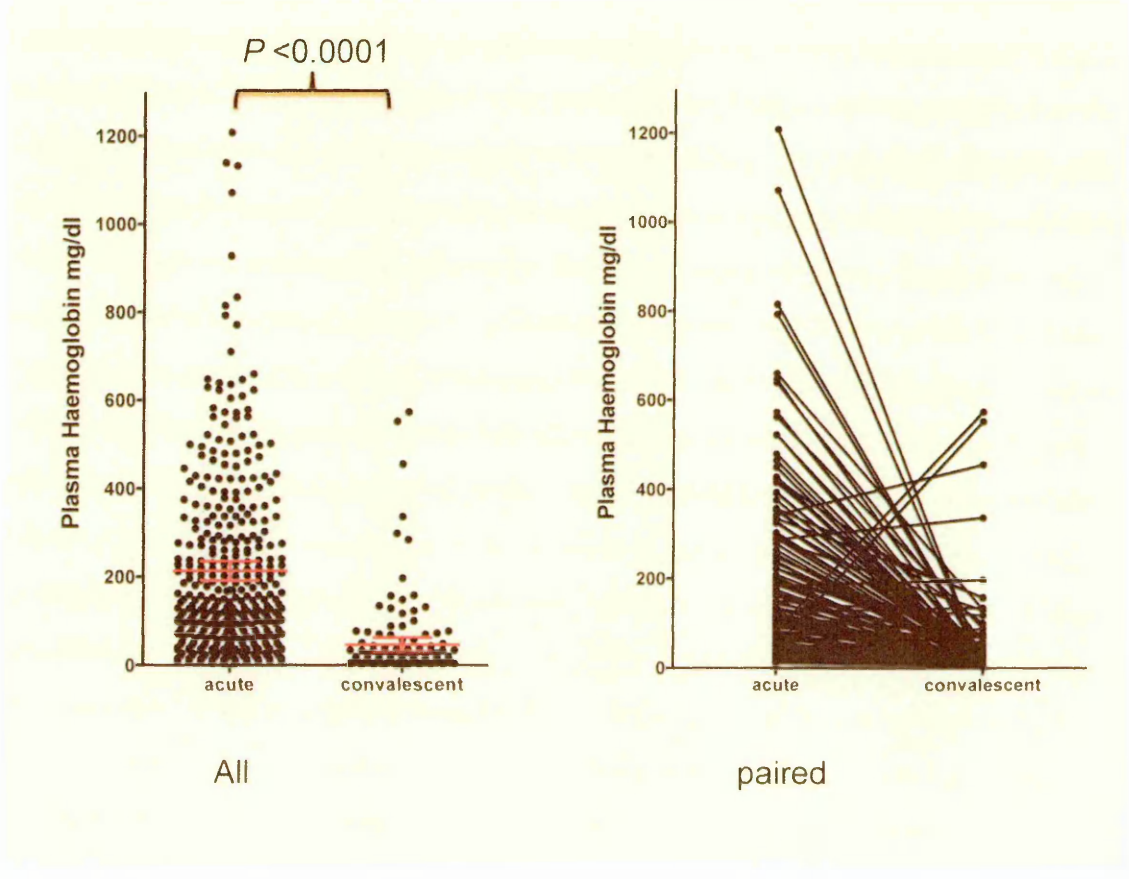


Figure 6.3. *Showing a higher mean plasma haemoglobin concentration among acute cases of DUS compared to convalescents (left) and on the right graphical representation showing a downward trend of plasma haemoglobin from acute to convalescent states.*

6.5.1.2. Urine Analysis

Of the 268 urine samples collected, 165 (61.6%) were randomly selected and analysed for urine proteins as described by O'Donnell *et al* in PNG [185]. We found haemoglobinuria in 92/165 (55.8%) and myoglobinuria in 59/165 (35.8%). There was evidence of high concentration of haemoglobin in urine among acute cases compared to convalescent cases $P<0.0001$ (Figure 6.4). Urine myoglobin was also high among acute cases compared to convalescents $P<0.0001$ (Figure 6.5).

Figure 6.4. Haemoglobin concentrations in urine samples collected from children with DUS at admission and during convalescence

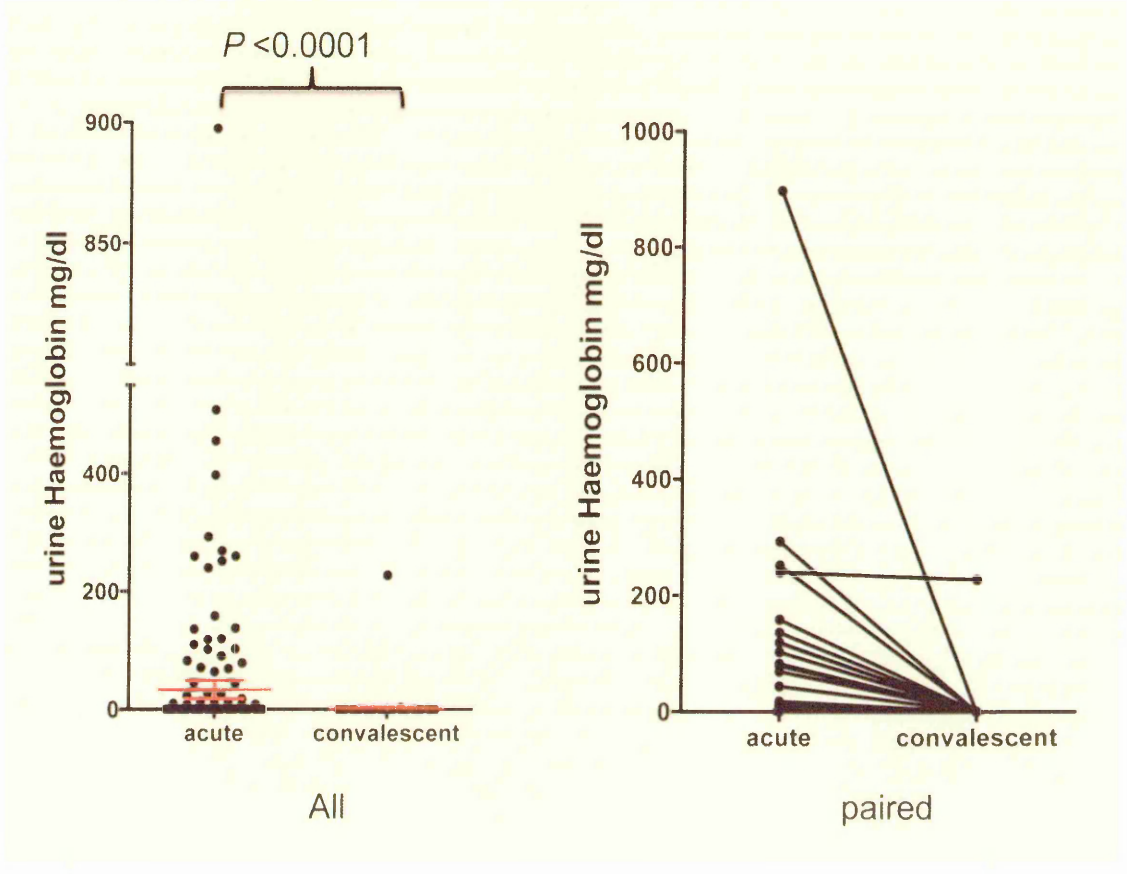


Figure 6.4. *Showing high level of urine haemoglobin indicative of massive haemolysis among DUS cases compared to their convalescent states*

Figure 6.5. Urine myoglobin between the acute and convalescent samples

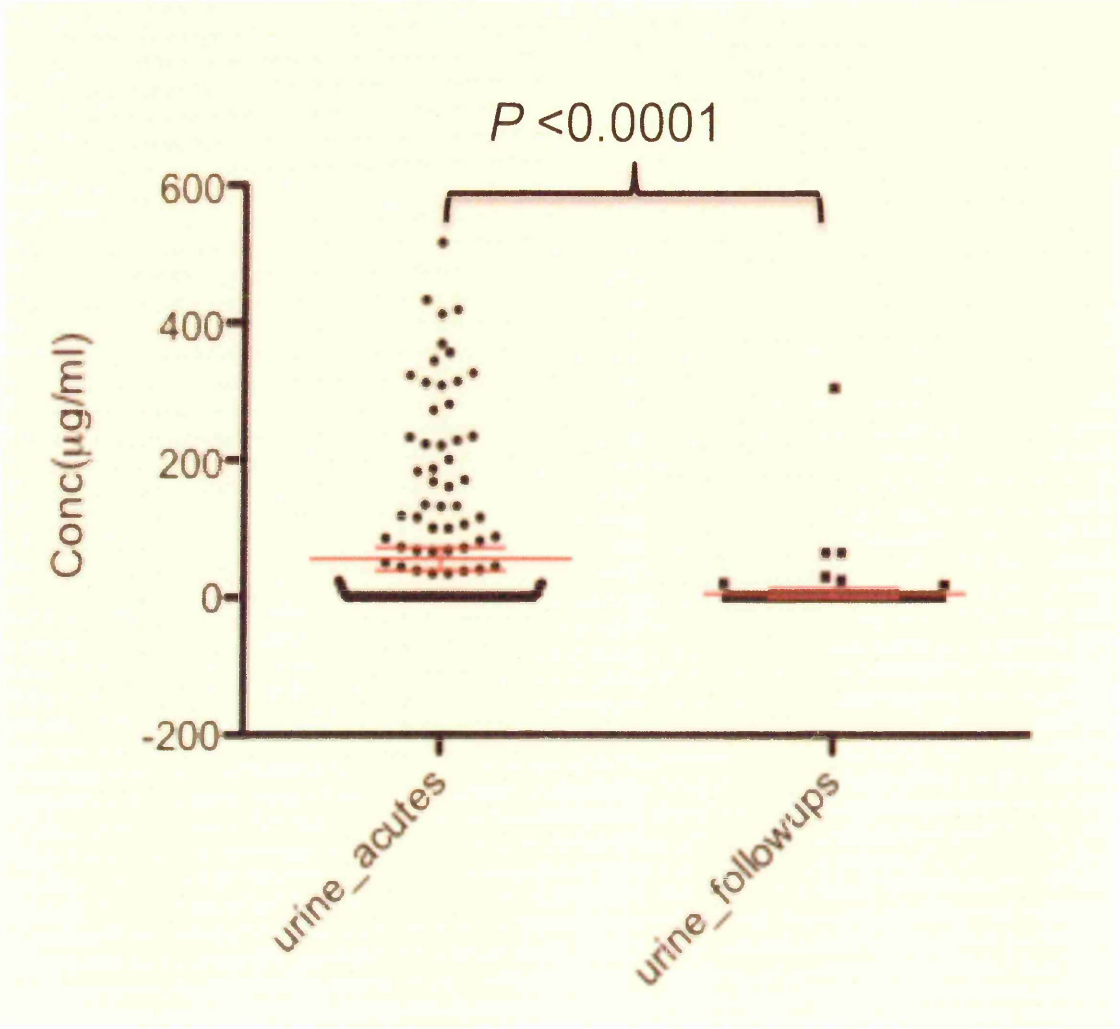


Figure 6.5. *Showing a higher proportion of DUS patients had urine myoglobin indicative of muscle injury compared to their convalescent states.*

6.5.1.3. Comparison of laboratory parameters between acute and convalescent samples

Further comparisons of laboratory parameters between the acute and convalescent samples were done. Parameters that were of significance were: malaria among acute in 24.6% v 10.9%; P=0.002, median Hb among convalescents 10.7 (IQR 9.6, 12.1) v acute 5.1 (IQR 3.9; 8.0) P<0.001. The proportion with thrombocytopaenia was high 82 (30.6%) among acute patients compared to 7 (7.8%) among the convalescent. The median reticulocytes among the acute cases were higher 2.9 (IQR 1 - 4.4) compared to 0.4 (IQR 0.1, 1.6) P=0.005. Summary of these laboratory parameters are in Table 6.3.

Table 6.3. Comparison of laboratory parameters between acute and convalescent samples

	Acute		Convalescent		*P-value*
	N = 268	Median (IQR) /Proportion [%]	N = 148	Median (IQR) /Proportion [%]	
Malaria slide positive N (%)	268	66 (24.6)	148	11 (10.9)	0.002
Haemoglobin g/dL, median (IQR)	268	5.1 (3.9 – 8.0)	93	10.7 (9.6 – 12.1)	<0.001
Severe anaemia (<5g/dL) N (%)	268	127 [47.4]	93	2 [1.4]	<0.001
Platelets (median, IQR)	227	190 (119, 281)	90	293 (201, 365)	<0.001
Thrombocytopaenia	227	82 [30.6]	90	7 [7.8]	-
Reticulocytes (median, IQR)	141	2.9 (1 – 4.4)	103	0.4 (0.1 – 1.6)	0.005
Plasma					
Haemoglobin mg/dL, median (IQR)	247	146.4 (63.8 –287.7)	93	11.6 (5.86 – 43.9)	<0.001
Haptoglobin mg/dL, median (IQR)	247	0.07 (0.02 – 0.31)	96	0.52 (0.07 – 1.01)	0.001
Myoglobin ng/mL (median, IQR)	234	57.8 (34.1 – 125.9)	68	46.4 (25.4 – 64.1)	0.952
Methaemoglobin µg/mL(median, IQR)	116	2.1 (0.9 – 3.7)	68	2.2 (1.1 – 4.1)	0.952
Urine					
Haemoglobin mg/dL (median, IQR)	143	1 (0 – 1)	92	0 (0 – 0.2)	0.002

Legend: *Numbers are N with proportions in parentheses, P-value from x^2, CI = confidence interval*

6.6. Drug exposure among patients with DUS

My case report form included questions about drug exposure among patients with DUS during the 48-hour period prior to admission. I classified drugs into: pharmaceutical drugs (including antimalarials and antibiotics), herbs (traditional concoctions usually of plant origin used for treatment of illnesses in the community) and blood transfusions.

Among the antimalarials, 129 (48.1%) had taken CoArtem (Artemether/Lumefantrine combination). This is the first large study linking CoArtem to DUS among African children. Before this, Aloni *et al*, in DRC linked this drug to DUS in an 8-year-old boy [360]. Nonetheless, case reports involving Artemesinin based combination have been reported especially in Asia [324]. Exposure to Quinine was found in 119 (44.4%) similar to some reports in African children with DUS in DRC [179]. The surprising finding was that of use of chloroquine among 26 (9.7%) children because chloroquine is not in the treatment guidelines except for prophylaxis in SCA. Malaria is known to be resistant to this drug and the resistant strains are responsible for severe and complicated malaria. Cotrimoxazole was used by 101 (37.7%) of children with DUS. Children who used at least two drugs (antimalarial and /or antibiotics) were 73 (27.2%) and at least 25 (9.3%) used three drugs. Herbal use was documented among 52 (19.4%) children while blood transfusion in the current illness was among 11 (4.1%). We could not confirm blood group incompatibility in this group of patients. Table 6.4 below summarizes exposure to drugs.

Table 6.4. Treatment received within 48 hours prior to admission as reported by caretakers of patients

Category	Drug/treatment	N/268 (%)	Survivors N (%)	Deaths N (%)	Relative risk (95%CI)	*P-value*
Antimalarials	Co-Artem	129 (48.1)	115 (48.5)	14 (45.2)	0.89 (0.46 – 1.72)	0.725
	Artemether	38 (14.2)	30 (12.7)	8 (25.8)	2.11 (1.02 – 4.36)	0.048
	Chloroquine	26 (9.7)	22 (9.3)	4 (12.9)	1.38 (0.52 – 3.63)	0.522
	Fansidar	28 (10.4)	22 (9.3)	6 (19.4)	2.10 (0.92 – 4.58)	0.085
	Quinine	119 (44.4)	103 (43.5)	16 (51.6)	1.34 (0.68 – 2.59)	0.390
Drug combinations	Any two antimalarial drugs above	73 (27.2)	64 (27.0)	9 (29.0)	1.11 (0.48 – 2.53)	0.812
	Any three drugs above	25 (9.3)	21 (8.9)	4 (12.9)	1.52 (0.48 – 4.77)	0.467
Antibiotics	Cotrimoxazole	101 (37.7)	86 (36.3)	15 (48.4)	1.6 (0.80 – 2.99)	0.191
Herbs	Herbal use	52 (19.4)	48 (20.3)	6 (19.4)	0.99 (0.43 – 2.30)	0.994
Blood	Blood transfusion	11 (4.1)	10 (4.2)	1 (3.2)	0.78 (0.12 – 5.20)	0.793

Legend: *Numbers are N with proportions in parentheses, P-value from x^2, CI = confidence interval*

6.7. Community distribution of DUS

To precisely locate cases and mortality due to DUS, I adopted the use of geographical position system (GPS) to generate data on community distribution of dark urine syndrome and determine the association between clinical severity and outcomes with geospatial data.

Most cased and deaths were in children from rural areas and marshlands in the Eastern Region (Figure 6.8).

Figure 6.6. Community distribution of DUS cases and deaths in eastern Uganda

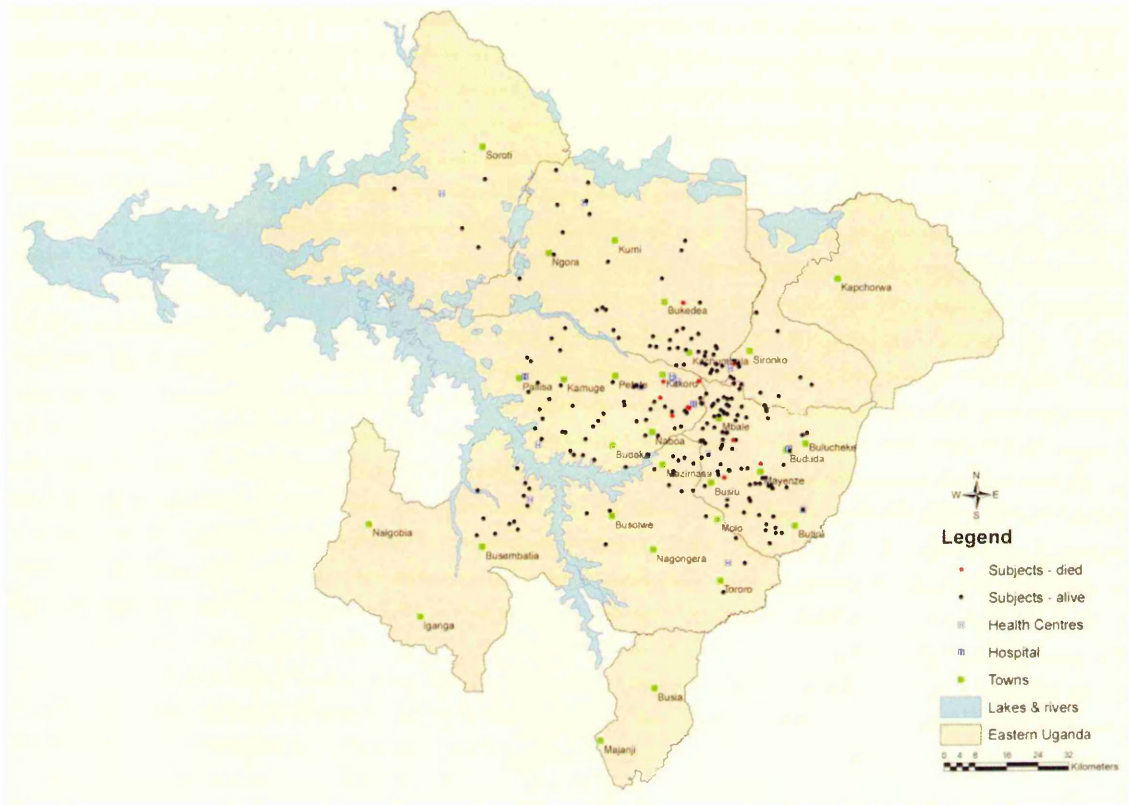


***Figure 6.6**. Showing GPS plots of DUS cases distributed away from urban centres and hospitals. The black dots indicate alive cases while the red dots indicate deaths in the community following discharge from the Mbale RRH.*

6.8. Risk factors for mortality

In a bid to explore which clinical features among children presenting with DUS were associated with particularly poor outcomes, I compared the prevalence of a range of clinical and laboratory parameters among cases who survived and those who died. Nine clinical features of severity were identified in the general demographic, clinical and laboratory characteristics. The risk of these clinical features for mortality was assessed at logistic regression and only those with $P \leq 0.05$ were included in the multivariate model. On univariate analysis significant variables included: pallor $P=0.050$, hypoxia $P=0.025$, CRT >2sec $P=0.042$, impaired consciousness $P<0.001$, prostration $P=0.003$, coma $P=0.001$, severe lactataemia $P<0.001$ and severe anaemia $P=0.003$. At multivariate model, however, no variable was independently associated with high risk of mortality (Table 6.5)

Table 6.5. Logistic regression analysis for predictors of mortality among patients with confirmed dark urine syndrome

Category	Parameter	ULR		MLR	
		Odds ratio (95% CI)	*P-value*	Odds ratio (95% CI)	*P-value*
General clinical features	Pallor	7.42 (0.97 – 55.81)	0.05	3.73 (0.47 – 29.94)	0.215
Respiratory	Hypoxia	4.24 (1.19 – 15.02)	0.025	2.36 (0.41 – 13.54)	0.335
Cardiovascular	CRT >2 sec	2.19 (1.02 – 4.67)	0.042	1.21 (0.48 – 3.01)	0.448
Nervous system	Impaired consciousness	11.88 (2.98 – 47.22)	<0.001	-	-
	Prostration	8.83 (2.04 – 36.49)	0.003	-	-
	Coma	6.08 (1.99 – 18.49)	0.001	-	-
Laboratory	Severe lactataemia	4.55 (2.10 – 9.88)	<0.001	2.18 (0.87 – 5.49)	0.097
	Severe anaemia	3.67 (1.58 – 8.55)	0.003	2.04 (0.77 – 5.43)	0.153

Legend: *P-value for univariate logistic regression (ULR) is from x^2 test, P-value for multivariate logistic regression (MLR) is from standard normal distribution, CI = confidence interval.*

6.9. Red cell genetic polymorphisms among children with DUS in Eastern Uganda

To explored the innate risk factors for DUS among children in Eastern Uganda. A case - control study was done. I found, sickle cell anaemia (HbSS) was a risk factor P=0.001, while sickle cell trait (HbAS) was protective against DUS P<0.001. The other innate characteristics studied included G6PD and alpha-thalassaemia, however, they were not statistically associated with increased risk of developing DUS (Table 6.6).

Table 6.6. Case-control study of genetic characteristics in dark urine syndrome

Category	Characteristics	Cases, N (%)	Controls, N (%)	OR (95% CI)	*P-value*
Number of samples	Number	268 (100.0)	494 (100.0)	-	-
Sex	Female	172 (64.2)	186 (37.6)	0.92 (0.68 – 1.26)	0.617
	Male	96 (35.8)	308 (62.4)	1.08 (0.79 – 1.49)	
alpha-thalassaemia	Homozygous	10/264 (3.8)	27/493 (5.5)	0.67 (0.32 -1.43)	0.312
	Heterozygous	98/264 (37.1)	183/493 (37.1)	0.97 (0.71 – 1.33)	0.458
	Normal	156/264 (59.1)	283/493 (57.4)	1.07 (0.78 – 1.47)	0.654
Sickle cell	HbSS	19/263 (7.2)	5/492 (1.0)	7.58 (2.69 -26.23)	0.001
	HbAS	11/263 (4.2)	69/492 (14.0)	0.04 (0.01 – 0.14)	<0.001
	HbAA	233/263 (88.6)	418/492 (85.0)	0.15 (0.05 – 0.40)	0.281
G6PD	Deficient	36/260 (13.8)	53/491 (10.8)	1.32 (0.84 – 2.09)	0.219

Legend:

Numbers are N with proportions in parentheses, denominator indicated where data are missing, P-value from x^2 test, CI = confidence interval, OR= Odds ratio

6.10. Discussion

In this study, I assessed the clinical, haematological, innate, infectious, drug exposure and community risk factors for DUS. This was the first attempt to achieve this in Eastern Uganda, an area with high prevalence of DUS in East Africa.

In this study I refined the definition to include history of dark urine as entry criteria, HCC as a screening criteria and physician confirmation of dark urine as a definitive criteria. In the current descriptions of DUS in children, various study populations have been described for single or dual aetiology usually malaria infection, exposure to quinine or G6PD [167, 179, 180, 190]. No study has comprehensively studied clinical, haematological, innate, infectious, drug exposure and community distribution in the same study. Even as I recognized that some exposures may work synergistically and that the pathophysiology of DUS is complex, for the purpose of this description I will discuss the associations of each factor one at a time. A majority of the patients were from rural areas; more proximal to the marshlands compared to urban areas a fact that may explain the differences in exposure to risks for DUS. In addition, delays in accessing appropriate treatment may have exposed children with malaria to disease progression. Furthermore, attempts of giving home therapies including herbal or manufactured antimalarials; some of which have toxic or oxidative characteristics is higher.

Rainy seasons have recently been associated with DUS. I found high prevalence of DUS coincided with the two peak rainy seasons in the region (May - August and November - January) (Chapter 3). However, this coincidence is more likely because it

is the peak seasons for malaria, therefore, intense transmission of malaria may have contributed to DUS in this population, just as was reported in DRC [190]. Patients were screened for infection at two levels: Clinically for acute respiratory tract infections and in the laboratory for *P. falciparum*, and *S. haematobium*. Thirty (11.2%) of the patients had pneumonia, none had *S. haematobium* and malaria was found in 66 (24.6%) and evidence of recent malaria infection using HPR-2 in 53.0%. Whereas, the pathophysiology of DUS with current malaria infection can be explained [361], the mechanism in slide negative or low parasitaemia remains poorly described. Moreover, timing of infection and clinical disease has puzzled current understanding of pathophysiology of haemolysis and later severe anaemia [116, 127, 128]. *P. falciparum* predominates in DUS, however, there are reports on mixed *P. falciparum* with *P. malariae*. In addition *P. vivax* induced DUS has been described in case studies, especially in severe immunosuppression [362]. For long the association between quinine and DUS has been known including its plausible temporal causative association [170, 179, 190]. In addition, other aryl-alcohol derivatives including halofantrine and mefloquine have been incriminated [363, 364]. In this study 129 (48.1%) patients had taken CoArtem® (Artemether/Lumefantrine combination). This is the first large study linking CoArtem® to DUS among African children. Before this, Aloni *et al* in DRC linked this drug to DUS in an 8-year-old boy [360]. The role of genetics in aetiology of DUS has long been well described [354], especially in G6PD deficiency [170, 176, 202, 355, 356] and sickle cell anaemia [357]. These data showed that the male: female ratio of 1.8: 1 makes G6PD deficiency as an unlikely major

underlying genetic aetiology. G6PD deficiency was found in 36/260 (13.9%) though we did not know the local community prevalence of this condition. The case control study too did not show increased risk of DUS among patients with G6PD deficiency. Of other structural red blood cell membrane defects, the prevalence of elliptocytosis was 32 (11.9%) and spherocytosis in 32 (11.9%). Sickle cell anaemia (HBSS) was risk factor for DUS P= 0.001 and sickle cell trait was a protective factor P<0.001. The role of immunity in DUS has not been well described. These results show that the children with DUS are older and coming from high malaria endemic areas suggesting break-through malaria infection. Some recent reports suggested that during seasons of high malaria transmission, children who are immune to malaria tend of develop DUS [190]. Use of herbal medication and fava beans have been implicated in the causation of DUS. Fifty two (19.4%) of the patients use herbal medication though the risk of developing DUS in these patients is difficult to determine.

The urine analysis indicated the presence of both haemoglobinuria 92/165 (55.8%) and myoglobinuria 59/165 (35.8%), similar to findings by earlier researchers on DUS in children in PNG [185]. However, the mechanism involved is not fully understood but may be due to a combination of pathways in malaria pathogenesis [185, 365-367]. In this study, pallor 220 (82.1%) and clinical jaundice 206 (76.9%) were common clinical manifestations both of which indicate underlying haemolysis in DUS, similar to findings from DRC [190].

This study, however, had limitations. Laboratory data were incomplete; renal and liver function tests, LDH assay and quantification assays for drugs were not done.

Despite these limitations, I have been able to demonstrate that DUS in children has wide range of aetiological factors.

Given this wide aetiology, at clinical interface on admission, it is not possible to differentiate children who present with haemoglobinuria from those who have myoglobinuria or haematuria. Before aetiology is confirmed I propose the use of the term dark urine syndrome (DUS) as was first used in PNG in 2006 [185].

In conclusion, DUS in African children is complex but I have been able to demonstrate that it is most likely an acquired phenomenon, with a wide range of risk factors depending on the study population and geographical location. The risks or associated factors include higher median age, malaria peak season, exposure to quinine and artemesinin based combinations, malaria infection, and sickle cell anaemia.

Chapter 7: Discussion

In this thesis I have used a number of approaches ranging from literature review, survey methods, clinical, laboratory to relevant data analyses to answer two research questions. Firstly, the burden and clinical spectrum of severe malaria and secondly the prevalence, complications, aetiology and outcomes of dark urine syndrome (DUS) among children admitted to the paediatric acute care unit (PACU) at Mbale Regional Referral Hospital (Mbale RRH).

The current literature indicates gaps in the descriptions of the clinical spectrum of paediatric severe malaria in Eastern Uganda. In addition, there were few data on childhood DUS in African children presenting to hospital. Furthermore, current series on adult type severe malaria [181, 182], and DUS differ in a number of ways from those observed in paediatrics [61, 180-182].

Given the fact that there was no baseline information on these two conditions in Eastern Uganda, it was important to conduct paediatric ward admission surveillance as an entry point for describing severe malaria and DUS presenting to the PACU.

The paediatric ward admitted young children with a median age of 16.0 months with over 87% of admissions being in the under 5years old, typical of malaria high transmission endemic areas [8]. Tropical infectious diseases were predominant among the admissions and as expected they commonly presented with non-specific symptoms including fever, cough, vomiting and diarrhoea; which mirrored the

common diagnoses of malaria, pneumonia and diarrhoea. The overall in-hospital mortality was high 655 (6.4%); similar to one study in Monrovia, Liberia (6.4%) [261] and the series in Kenya hospitals [245], but was much lower than the rates from another study in Liberia (13.1%) [262] and the other in Zimbabwe (17.8%) [320]. In my ward surveillance study (WSS), the neonates (<1 month) had poor mortality outcomes with case fatality rate of 13.2% compared to children >1month at 3.7%, P=0.0001, indicating heavy burden of disease in the neonates. Conversely, my data may point towards poor emergency care in these settings since early mortality was observed. My results indicated that the number one cause of admission was malaria, followed by pneumonia and were consistent with reports on burden of disease in Uganda [290, 298]. As expected, in this malaria endemic region, there was a strong relationship between severe anaemia and malaria; P<0.001, with most of the children with severe anaemia being infants (median age 12.0 months); a feature that has been described in malaria endemic areas [8, 113, 118].

Dark urine based on clinical history as earlier defined by other researchers [183, 190, 324, 325], was also common 1,087 (10.6%) in my study. But in addition, for the prospective surveillance of DUS, I refined the final definition to include history of dark urine as entry criteria, HCC as a screening criteria and physician confirmation of dark urine as a definitive criteria. Studies on aetiology of dark urine in infants point to a haemolytic process due to an inherited genetic abnormality such as G6PD [202] or ABO blood incompatibility. Among the older children, however, in addition to some genetic aberration, acquired phenomenon including infections and autoimmune

phenomenon have been implicated [326]. These have been described in detail in my thesis chapters 5 and 6.

In total, 662 children were recruited into severe malaria surveillance and described in detail in Chapter 4. The median age was 18months (IQR 10; 33), and without any surprises the majority 602 (91.9%) of children were under 5 years of age suggesting a role of poor level of acquired immunity and indicating malaria high transmission background. The common clinical features of paediatric severe malaria in these settings were respiratory distress 554/662 (83.7%), shock 411/662 (62.1%), clinical jaundice, severe anaemia, hyperlactataemia and DUS. The overall case fatality rate in severe malaria was high 63/662 (9.5%) and the case fatality rates increased with multiple combinations of clinical manifestations; consistent with earlier series in Kenya [8], The Gambia [216, 339, 340], Malawi [21], and Sudan [341]. Complicated respiratory distress had higher case fatality rates and so was complicated severe anaemia. These complications were associated with worsening metabolic and major organ functioning with poor outcomes as we previously described [342]. In severe malaria, the WHO shock criteria (CRT >3seconds, weak and fast pulse) [254]; could only identify few children 6/662 (0.9%), however, these were associated with worse outcomes [mortality rate 2/6 (33.3%)]. My study found a high prevalence 177/662 (26.7%) of clinical jaundice in children with severe malaria contrary to other series in which jaundice was rarely reported in children [1, 8]. My findings may be of unique relevance to the populations in Eastern Uganda since severe anaemia and jaundice are both frequent in my study and indicators of massive haemolysis. My findings

varied from other series from low transmission areas with older median age and frequent neurological manifestation [22, 24, 225].

Severe malaria complicated by renal failure has not featured frequently in earlier paediatric descriptions. This is possibly due to a number of reasons, including the nature of rapid progression of severe malaria disease with deaths occurring early in the disease before identification of renal impairment. In the recent times, however, some studies have reported acute renal failure (ARF) in severe malaria in African children. This is particularly in children with DUS [179, 180]. In my study, 14% (n = 662) of the study subjects with severe malaria had DUS. Unlike studies in Nigeria and DRC, none of the patients in my study presented with history suggestive of acute renal failure, even though on clinical history alone, one cannot exclude renal impairment. Findings from a large randomised trial, AQUAMAT conducted in 10 sites in 9 countries in Africa involving 5,425 children across a wide range of transmission intensities indicated that haemoglobinuria was present in only 237/5,426 (4.5%) with similar (9%) mortality to those without haemoglobinuria (10%) [61]. Furthermore, the authors noted that clinical evidence of jaundice was rare, present in only 114 (2%) but this feature was associated with a significantly higher mortality (19% versus 10% in those without jaundice) [61]. In my series, three clinical features were independently associated with increased risk of mortality, including: hypoxia (P=0.007), severe anaemia (P=0.028) and severe lactataemia (P=0.001). Hypoxia has not been frequently reported in other series.

The data from the FEAST trial (Chapter 5) provided an opportunity to study the geographical distribution and clinical features of the DUS phenomenon in East Africa and its relationship with malaria endemicity across sites. I found that DUS was common 394/3,170 (12.4%) among FEAST study participants. There was marked geographic variation; with the majority of DUS patients 318/394 (81.0) presenting to sites in Eastern Uganda, suggesting the role of high malaria transmission though only 147/300 (49.0%) had malaria parasitaemia (or evidence of current infection). Although *P. falciparum* was less prevalent on malaria microscopy among the DUS patients than NDUS, a higher proportion of cases were positive for HRP-2, a marker of recent malaria infection. Moreover, HRP-2 concentrations were significantly lower among DUS than NDUS, observations that together suggest that many of the children with DUS had received recent antimalarial treatment and associated with a delayed onset of haemolysis. Use of herbal medication and fava beans have been implicated in the causation of DUS. Fifty-two (19.4%) of the patients used herbal medication though the risk of developing DUS in these patients is difficult to determine.

Epidemiologically, the older median age of patients presenting with DUS in high malaria endemic areas and the lack of male sex predominance would suggest that neither malaria nor glucose-6-phosphate dehydrogenase deficiency respectively were the major underlying causes of this condition. The common triad of this syndrome included dark urine complicated by clinical jaundice in 256/318 (80.5%) and severe anaemia (Hb <5g/dL) in 238/310 (77.0%). These complications are indicative of underlying acute severe haemolysis. The urine analysis indicated the presence of both

haemolysed (54%) and non-haemolysed blood (11%) and bilirubin (22%) suggestive of both pre-renal and renal contribution of DUS in my study patients, but may not be exclusive to these groups alone. Moreover, clinical jaundice, severe acidosis and evidence of kidney injury were more common in children with DUS. The 48-hour and Day-28 mortality were similar in children with and without DUS. These data do not support a single haemolytic trigger but point at a possibility of multifactorial causes including infections especially malaria, innate characteristics and oxidative agents (especially drugs, herbs and diet). In chapter 6 similar to a prospective study in Papua New Guinea [185], in which 351 children were studied for the causes of dark urine 22 (6.3%), two proteins haemoglobin and myoglobin were found in these patients indicating two unrelated aetiologies of dark urine namely: massive haemolysis and muscle breakdown respectively. These findings may suggest that my DUS series had one or both proteins responsible for dark urine. Indeed the urine analysis indicated the presence of both haemoglobinuria 92/165 (55.8%) and myoglobinuria 59/165 (35.8%), similar to DUS in children in PNG [185]. However, the mechanism involved is not fully understood but thought to be a combination of pathways in malaria pathogenesis [185, 365-367]. The evidence implicating malaria in the causal pathway is in keeping with historical reports and recent reports in children [172, 190] and involving recent anti-malarial treatment. For long the association between quinine and DUS has been known including its plausible temporal causative association [170, 179, 190]. In addition, other aryl-alcohol derivatives including halofantrine and mefloquine have been incriminated [363, 364]. In this study 129 (48.1%) patients had

taken CoArtem (Artemether/Lumefantrine combination). This is the first large study linking CoArtem to DUS among African children. Before this, Aloni *et al* in DRC linked this drug to DUS in an 8-year-old boy [360].

Finally, the prevalence of two well-documented malaria-protective traits [359] HbAS and alpha-thalassaemia was significantly lower in DUS than in community controls. The protective association between these traits and DUS also implicates malaria in its aetiology within our study population. Of other structural red blood cell membrane defects the prevalence of elliptocytosis was 32 (11.9%) and spherocytosis in 32 (11.9%). Sickle cell anaemia (HBSS) was risk factor for DUS P=0.001 and sickle cell trait was a protective factor P<0.001.

Despite lower rates of recovery from severe anaemia at 48 hours, DUS patients by day 28 had a higher Hb compared to controls patients (P<0.001). This may be twofold, firstly, a lower malaria infection rate at day 28 of follow up and secondly, higher transfusion rates as reported in observations (Olupot-Olupot and Engoru, 2007-2012 up) may have resulted in a superior bone marrow recovery.

The prevalence of HIV and its case fatality rate were low, despite a general national increase in prevalence of HIV from 6.3% before 2010 to 7.3% in 2011; it is even higher in internally displaced people's camps in the northern part of the country [328].

This study, however, had limitations. Laboratory data were incomplete; renal and liver function tests, LDH assay and quantification assays for drugs were not done. Despite these limitations, I have been able to demonstrate that DUS in children has

wide range of aetiological factors and that both recent and current malaria are associated with the phenomenon.

Given this background on aetiology, at clinical interface on admission, it is not possible to differentiate children who present with haemoglobinuria from those who have myoglobinuria or haematuria. Before aetiology is confirmed I propose the use of the term dark urine syndrome (DUS) as was first used in PNG in 2006 [185].

These data, findings and conclusions present a baseline on which I intend to pursue further research on the following research areas:

I. The role of artemesinin based combination drugs in delayed haemolysis.

II. Genome wide association studies of severe childhood malaria in eastern Uganda.

III. Community studies on risk factors and control of DUS in Eastern Uganda.

IV. Immunological profile of DUS.

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Appendices

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THE REPUBLIC OF UGANDA

MINISTRY OF HEALTH
MBALE REGIONAL HOSPITAL
P.O. BOX 921
Mbale – Uganda

In any correspondence on this Subject, please quote: REIRC 005/2010

Date 5th March 2010

MRHIRC ACCREDITED BY THE UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Attn: Dr. Peter Olupot – Olupot
Mbale Regional Referral Hospital
C/o P. O. Box 1966, Mbale

Dear Dr. Peter Olupot – Olupot

RE: ETHICAL REVIEW OF THE STUDY

At the sitting of the Mbale Regional Hospital Institutional Review Committee (MRHIRC) on the 4th March 2010 to review your study, the committee was satisfied with the protocol and responses to the issues raised.

However, on the shipment of the study samples to the laboratory at KEMRI/Well come Trust, Kilifi Kenya; there is need to obtain clearance for shipment of samples from Uganda National Council of Science and Technology (UNCST) after you have collected the samples.

I am pleased to let you know that during the meeting, the committee in **Min. 9:2** of the MRHIRC meeting on 4th March 2010 cleared your study "***The burden of Spectrum of Paediatric Severe Malaria and Aetiology of Haemoglobinuria in Eastern Uganda, version 1.0, January 2010"***

The study may proceed as scheduled. This study clearance letter is valid for the period March 4th 2010 – March 3rd 2011. In the event that the study is not complete by then, you are obliged to seek clearance for extension of the study from the MRHIRC in writing. In the mean time you are required to avail with progress reports of your study to MHRIC at 3 monthly intervals.

Thank you

Yours sincerely

Dr. Crispus Tegu
Chairman, MRHIRC

MBALE REGIONAL HOSPITAL
INSTITUTION REVIEW COMMITTEE
P.O. BOX 921 MBALE
APPROVED
Date: 5/03/2010

c.c MRHIRC File

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THE REPUBLIC OF UGANDA

MINISTRY OF HEALTH
MBALE REGIONAL HOSPITAL
P.O. BOX 921
Mbale – Uganda

In any correspondence on this
Subject, please quote: REIRC 011/2011

Date 27TH April 2011

MRHIRC ACCREDITED BY THE UNCST, REGISTRATION NUMBER IRC 019

MBALE REGIONAL HOSPITAL 27 APR 2011

Attn: **Dr. Peter Olupot-Olupot**

Principal Investigator

Dear Dr. Olupot-Olupot

RE: ETHICAL CLEARENCE FOR THE STUDY "THE BURDEN & SPECTRUM OF PAEDIATRIC SEVERE MALARIA AND AETIOLOGY OF DARK URINE(BLACKWATER FEVER) IN EASTERN UGANDA

On behalf of the Mbale Regional Hospital Institutional Review Committee (MRHIRC) I am pleased to let you know that your progress report was reviewed and found satisfactory and that your request for renewal of the ethical clearance for the study entitled **"The Burden & Spectrum of Paediatric Severe Malaria and Aetiology of Dark Urine (Blackwater fever) in Eastern Uganda"** was granted.

This ethical clearance runs for the period (5th May 2011 – 4th May 2012).

You are required to submit 6 monthly progress reports to the MRHIRC. Should you make any changes to the protocol, please apply for the ethical clearance of the amendments with the MRHIRC.

Scheduled monitoring will be done at the research site by MRHIRC to monitor adherence to the approved study protocol.

You are required to register your study with the Uganda National Council for Science and Technology.

On behalf of the Mbale Regional Hospital Institutional Review Committee (MRHIRC), I wish you the best of luck in your study.

Sincerely

Dr. Crispus Tegu
Chairman
MRHIRC

27/04/11

MBALE REGIONAL HOSPITAL
INSTITUTION REVIEW COMMITTEE
P.O.BOX 921 MBALE
APPROVED
Date:.....

Mbale Regional Referral Hospital Paediatric Admission Record

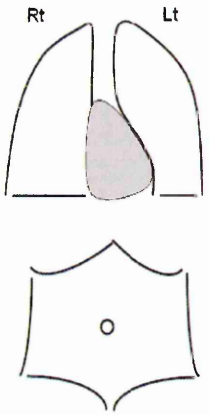
Name		Date DD/ MM/ YY	Age
IP No.		Time HH: MM (24hour)	Sex M / F DOB
Next of kin: Mother's Name Father's Name		Ethnic Group Ethnic Group	
Address/Name of Local Council (LC)		Details	Telephone contact
Weight (Kg) [ ][ ].[ ]	**Temp (°C)** [ ][ ].[ ]	**Heart rate:** [ ][ ][ ]	**Oxygen sats** [ ][ ][ ] %
Vaccines: M'sles Y / N DPT x (doses) HiB Y / N BCG Y / N			
Height (cm) [ ][ ].[ ]	**Head circumference (cm)** [ ][ ].[ ]	**MUAC (cm)** [ ][ ].[ ]	

Presenting complaints:

History		
Length of illness **hours** **days** **weeks**		
Fever	Y	N
Cough	Y	N
Cough >3 weeks	Y	N
Difficulty breathing	Y	N
Diarrhoea	Y	N
Diarrhoea >14 days	Y	N
Diarrhoea bloody	Y	N
Vomits	Y	N
Can drink/breastfeed?	Y	N
Blood/tea coloured urine	Y	N
Previous admission with tea/dark urine	Y	N
Previous admission for transfusion	Y	N
Convulsions in this illness	Y	N
Convulsions lasting > 30 mins	Y	N
Does your child have epilepsy	Y	N
Does your child have other neurological problem **If yes state:**.....	Y	N
NEONATES and < 2 MONTHS		
Abnormal movements/behaviour	Y	N
High pitched cry	Y	N
Apnoea	Y	N

Examination					
Airway	**Clear**	**Stridor**	**Needs active support**		
Breathing	Respiratory rate	/min (O if arrested)			
	Central cyanosis	Y	N		
	Grunting	Y	N		
Respiratory distress	Indrawing	Y	N		
	Deep 'Acidotic' breathing	Y	N		
	Wheeze	Y	N		
	Crackles	Y	N		
Circulation	**Radial pulse**	Weak normal bounding			
	Cap refill X	<2	2-3	>3	
	Temperature gradient legs (cool to warm: feet to groin)	Y	N		
	Pallor/Anaemia (tongue)	0	+	+++	
Dehydration	Sunken eyes	Y	N		
	Skin pinch (sec)	0	1	2	
Disability	AVPU A	V	P	U	
	Conscious level: Alert ☐ Lethargic ☐ Prostrate (unable to sit/feed) ☐ Coma ☐				
	If convulsion now, > 30 mins	Y	N		
	Bulging fontanelle	Y	N		
	Stiff neck	Y	N		
General/	Jaundice	0	+	+++	

Pus + cellulitis, umbilicus	Y	N		**nutrition**	Visible severe wasting	Y	N
Other:					Oedema of Kwashiorkor	Y	N
Any other					Oral candidiasis	Y	N



Admission Diagnoses			
Malaria	☐ severe (AVPU<A, or Resp Dist) ☐ Non - Severe	**Anaemia**	☐ Severe ☐ Non - Severe
Pneumonia	☐ Very Severe ☐ Severe ☐Non-Severe	**Meningitis**	☐
Diarrhoea	☐Non-bloody ☐ Bloody	**Neonatal sepsis**	☐
Dehydration	☐Severe ☐ Some	**Birth asphyxia**	☐
HIV/AIDS	☐Known ☐Possible	**Prematurity /VLBW**	☐
Malnutrition	☐ Kwashiorkor ☐ Marasmus ☐Marasimic-Kwash	**Circulatory Shock**	☐
If severe malaria classify	☐ Cerebral Malaria ☐Hypoglycaemia ☐ Repeated Convulsions ☐ Respiratory Distress ☐ Haemoglobinuria ☐ Acidosis ☐ Jaundice ☐ Hypovolaemic Shock ☐ Renal Failure ☐ Severe Malaria Anaemia (SMA)		
If Dark/Tea coloured urine (Paroxysmal grading)			
Other Diagnosis 1			
Other diagnoses 2			

Investigations ordered and results (=)
Legend LP = Lumber puncture, BS = Blood Slide, Hb= Haemoglobinuria, B/culture = Blood culture, HCT = Haemacott, RDT = Rapid Diagnostic Test, CXR = Chest X – Ray, US = Ultra Sound

Malaria BS	Y	N	=	**Glucose**	Y	N	=
Malaria RDT	Y	N	=	**LP**	Y	N	=
Hb/HCT	Y	N	=	B/ culture	Y	N	=
CXR/US Scan				Other 2			

Treatment, supportive care & Observations-indicate what care is prescribe and sign please

Keep warm	☐	Oxygen	☐	**Additional notes**
IV Fluids Plan	☐	Blood Transfusion	☐	

ORS (oral pr ngt) ☐	☐	Nutrition Feeds plan	☐	
Medical Review		**Nursing observations Plan**		**Immunisations needed**
Re-assess within 6 hours ☐ Routine ☐		High priority ☐ Routine ☐		BCG ☐ OPV ☐ DTP /Penta ☐ Measles ☐
Outcome: DOA: DOD: Any complications noted at discharge? Y / N (if Yes, Specify.....)		Status at Discharge: Alive / Dead		Clinician's Name and Signature .....

INFORMATION SHEEFOR CARETAKERS AND THTUDY PARTICIPANTS

Study title: The Burden and Spectrum of Paediatric Surveillance in Eastern Uganda

Lead Investigator: Dr. Peter Olupot-Olupot

Introduction

We encounter a number of patients with features suggestive of severe malarial illness admitted in Mbale hospital. This condition is noticed to be manifesting with fast or difficulty in breathing, impaired level of consciousness and severe anaemia. In this study we are trying to understand the burden and the spectrum of severe malaria among patients presenting for care at acute care unit at Mbale Hospital. with the aim of clearly describing the in-patient burden and features associated with severe and complicated malaria in our settings. This will also inform the clinicians the nature of burden and spectrum of disease they meet while executing day to day work and hopefully pave way to improved care and management of these patients.

What are we asking for?

We request you to give us permission to collect and store a small amount of your child's blood and urine, in order to do tests to find out what caused your child's illness. Some of the tests to find out what caused your child's illness are needed as part of this research cannot be done in Uganda at the moment, so part of the samples will be stored and sent to laboratories overseas.

Any other future research done on these samples, which is not part of this study, must first be approved by a national independent expert committee, to ensure that participants' safety and rights are respected. In addition we will look for you, if possible, so that you also give us permission to do the research.

Confidentiality.

The study that your child will be involved in has been approved by Mbale Regional Hospital institutional Review Committee and Uganda National Council for Science and Technology. Both are approval bodies in Uganda that examines suitability and safety of research studies involving human beings. Any information in this study will be kept confidential and is held on computers and coded so that only those concerned with research can access it.

Risks

The only risks are of momentary discomfort from blood sample collection for the tests to help treat your child.

Benefits

Your child will get no direct benefits from this study. However, we will ensure that we give your child the standard of care currently recommended by the Ministry of Health. And by taking part your child may help us improve the care of children who have serious illness in the future.

Participation

Your participation in this study is solely voluntary and that you can opt out at any time. However your refusal or opt out will not in any way affect the standard of care your child will receive.

If you have any questions or require further information, please contact

Dr. Petet Iupot-Olupot, Mbale regional Referral Hospital, P.O Box 921 Mbale, Uganda. Or call Tel. 0772 457217

Consent Form for the study, collection and long term storage of blood and Urine

The Burden and Spectrum of Paediatric Severe Malaria Surveillance in Eastern Uganda

This form should be completed after the patient information sheet has been read or read to

Child's Initials			

Male O Female O	Date/Year of Birth	D	D	M	M	M	Y	Y	Y	Y	Age	Y	Y	M	M

Date	D	D	M	M	M	2	0	Y	Y	Time				

I have read/been read to the information sheet for the Severe Malaria Surveillance study. I have understood everything and have had my questions answered satisfactorily. I understand that I may change my mind at any stage and that this will not affect the benefits due to my child.

I agree that my child participates in the study	Yes/No
I agree that small samples of blood and urine from my child may be kept by the FEAST team for studies related to this study, and any other further studies.	Yes/No
I understand that these results and samples will not be identified by either my or my child's name.	Yes/No
I agree to samples being exported overseas for further studies	Yes/No

Parent's/guardian's signature(or thumbprint)	Name	Date							
		D	D	M	M	M	2	0	Y	Y

Witness's signature (if thumbprint used above)	Name	Date								
		D	D	M	M	M	2	0	Y	Y

Investigator's'/designee's signature	Name	Date								
		D	D	M	M	M	2	0	Y	Y

FOR CHILDREN WHO PRESENT WITH FEATURES OF SEVERE MALARIA ADMITTED AT MBALE REGIONAL REFERRAL HOSPITAL.

Hospital Number |__|__|__|__|__|__|

Child's name _____ Child's initials |__|__|__|

Age |__|__|years & |__|__|month Sex: Male ☐ Female ☐

Weight |__|__|.|__|kg Height|__|__|__|.|__|cm MUAC |__|__|.|__|cm

Date of admission |__|__|/|__|__|__|/|__|__|__|__| Time of admission |__|__|:|__|__|

SCREENING DATA

Axillary temperature |__|__|.|__|°C

Respiratory rate |__|__|__|breaths/min

Oxygen saturation |__|__|__|%

Heart rate |__|__|__| beats/min

Conscious level ☐ alert ☐ prostrate ☐ coma

Fitting/convulsions at admission ☐ yes ☐ no

Systolic pressure |__|__|__|mmHg

Hypotension ☐ yes ☐ no

Capillary refill time ☐ seconds

a. Severe tachycardia ☐ yes ☐ no
b. Capillary refill time >2s ☐ yes ☐ no

c. Temperature gradient ☐ yes ☐ no
d. Weak pulse ☐ yes ☐ no

e. Impaired perfusion (YES if any one of a, b, c or d is YES) ☐ yes ☐ no
f. History of fever OR abnormal axillary temperature (>37.5°C or <36°C) ☐ yes ☐ no
g. Respiratory distress ☐ yes ☐ no
h. Impaired consciousness ☐ yes ☐ no
i. Passing dark/tea coloured urine ☐ yes ☐ no
j. Previous admission with dark/tea coloured urine ☐ yes ☐ no

Patient qualifies if f and g/or h are YES
Patient excluded OR entered into PBS if i &/or j are YES

INFORMATION KEY

a. Severe tachycardia = [<12m:>180bpm] OR [12m to 5y:>160bpm] OR [>5y:>140bpm]
e. Impaired perfusion = severe tachycardia OR capillary refill time >2s OR temperature gradient OR weak pulse.
d. Hypotension SBP =[<12m:<50mmHg] OR [12m to 5y:<60mmHg] OR[5y:<70mmHg]
g. Respiratory distress= deep breathing or increased work during breathing.
k. Prostration=inability to sit unsupported or breast feed if <9month & Coma=inability to localize a painful stimulus.

Investigator's Name_____Signature_____

Date |__|__|/|__|__|__|/|__|__|__|__|

Patient name _____Hospital Number |__|__|__|__|__|__|

Investigations

Haemoglobin result |___|___|.|___| g/dl

Lactate result |___|___|.|___| mmol/l

Glucose result |___|___|.|___| mmol/l

I-stat ☐ yes ☐ no

Urine Results.

Red blood cells	☐ Y	☐ N.....
Haemoglobinuria	☐ Y	☐ N.....
Bilirubinuria	☐ Y	☐ N.....
Urobilirubinuria	☐ Y	☐ N .....
Ketones	☐ Y	☐ N.....
Albuminuria (Protein)	☐ Y	☐ N.....
Nitrites	☐ Y	☐ N.....
Urine sugar	☐ Y	☐ N.....
Urine PH	☐ Y	☐ N.....
Specific gravity (SG)	☐ Y	☐ N.....
Urine leucocytes	☐ Y	☐ N.....
Red blood cell casts	☐ Y	☐ N.....

Check list.

Blood and urine sent to laboratory for storage.

Blood ☐ yes ☐ no

Urine ☐ yes ☐ no

Malaria slide ☐ yes ☐ no

Malaria slide Result _____

Malaria Parasite Density _____

Name of investigator: _____ Signature _____

Date: |__|__|/|__|__|__|/|__|__|__|__|

COUNTER SIGNED BY STUDY LABORATORY TECHNICIAN.

Name _____ Signature _____

Date |__|__|/|__|__|__|/|__|__|__|__| Time |__|__|:|__|__|

Study on Dark Urine among children admitted to Mbale Regional Referral Hospital in Eastern Uganda.

Name: _____ **(Initials)** _____ **IP No:** _____

Date of Admission: /D /D /M /M /M Y/ Y /Y Y/__/__/ Time: __ __. __ __ (24hrs)

Name of father _____ Tel: _____

Name of mother _____ Tel: _____

Locator details : L. C. 1 Zone: _____

L. C. 1 Chairman: _____

Village: _____

Landmark: _____

Sub – County: _____

District : _____

Telephone Contacts (2) _____

Date of Birth: ____/____/____ Age: _____ Sex: Male ☐ Female ☐

Anthropometry: Weight: _____ (kg) Height: _____ (cm)

MUAC: _____ (cm) Head circumference _____ (cm)

Immunization History Completed ☐ On schedule ☐ Defaulted ☐

Summary of Clinical Notes

*** History on dark Urine**			
When was the 1st episode of Dark Urine D D M M Y Y Y Y			
What age was the patient then _____._____ (months)			
How many episodes has child had _____._____			
*** Symptoms during this illness**		**Tick**	
Fever		Y	N
Cough		Y	N
Diarrheoa	Bloody Diarrheoa	Y	N
	Non bloody diarrheoa	Y	N
Vomiting	Vomits feeds	Y	N
	Vomits blood	Y	N
Yellow eyes		Y	N
Difficulty in breathing		Y	N
Bleeding from nose, ears or other body openings		Y	N
Micturation	More frequent than before onset of illness	Y	N
	Less frequent than before onset of illness	Y	N
	Painful	Y	N
	Passing very little urine than before	Y	N
	Passing more urine than before	Y	N
*** History of transfusion in this illness**			
Transfused in this illness (another hospital)		Y	N
Was episode of dark urine prior to blood transfusion?		Y	N
Was episode of dark urine after the blood transfusion?		Y	N
Has your child complained of severe muscle pain		Y	N
Is yes -are they not able walk/move owing to the pain		Y	N

*** History of drug use in last 14 days**		**Tick**	
Quinine		Y	N
Coartem (Artemeter/Lumefantrine)		Y	N
Artenam		Y	N
Primaquine		Y	N
Fansidar (Sulfadoxine/pyrimethamine)		Y	N
Chloroquine		Y	N
Cotromaxazole		Y	N
Others (Specify):			
*** Transfusion History**			
Any previous blood transfusions		Y	N
If yes, how many blood transfusions since onset of dark urine illness _____			
Were there any blood transfusion reactions during any of the blood transfusions		Y	N
When was the last blood transfusion			
	Within 72 hrs	Y	N
	Within 4 weeks	Y	N
	Within 3 months	Y	N
Dietary History/ Traditional medicines		Y	N
Name		Days before	
Herbal medicine taken in this illness			

Past medical and family History

- How many admissions has the child had due to passing dark urine in the last 12 months _____
- Family history of Sickle Cell Anaemia/ Disease Yes ☐ No ☐
- Is the patient a known 'sickler' (sickle cell disease) Yes ☐ No ☐
- How many siblings (brothers & sisters) does the patient have? Sister _____ Brothers _____
- If there are other children (siblings) with this kind of sickness, how many are females _____ males _____
- What is the birth order of the patient and other siblings with this condition: Patient _____ Others ___ ___ ___ ___ ___
- Are there other close relatives with this condition? Yes ☐ No ☐
- If yes to the above from paternal side ____ ____ ____. From maternal side ____ ____ ____

* Clinical findings	Tick						
Urine: Dark Urine	Y	N		Epistaxis		Y	N
Paroxysmal grading: Use Chart				Coma		Y	N
Fever	Y	N		Splenomegaly	Present	Y	N
Jaundice	Y	N			Size (cm BCM)		
Lymphadenopathy (generalized)	Y	N		Hepatomegaly	Present	Y	N
Anaemia (Pallor)	Y	N			Size (cm BCM)		
Severe Dehydration	Y	N		Heart	Tachycardia	Y	N
Petechial Haemorrhage (bleeding under the skin)	Y	N			Gallop rhythm	Y	N
					Added sounds/murmurs	Y	N

Patient name _____ Initials|___|___|___|

Investigations

Bedside tests

Haemoglobin result |___|___|.|___| g/dl

Lactate result |___|___|.|___| mmol/l

Glucose result |___|___|.|___| mmol/l

I-stat ☐ ☐

Checklist

Blood and Urine sent to lab for storage:

Blood ☐ ☐

Urine ☐ ☐

Malaria slide ☐ ☐

Name of investigator: _____ Date: _____/_____/_____

* Laboratory results—Lab results can be reported on a different form – we can design these later				
Specimen	**Test**	**Y**	**N**	**Result**
*** Blood**	**Purpose**	**Done (tick)**		
	Malaria parasites	Y	N	
	Parasite density	Y	N	
	Haemoglobin	Y	N	
	Sickle Cell Test	Y	N	
	Spherocytes	Y	N	
	Eliptocytes	Y	N	
	Reticulocytes	Y	N	
	Platelets	Y	N	
	White blood cell counts	Y	N	
	Eosinophils	Y	N	
	Neutrophils	Y	N	
	Lymphocytes	Y	N	
	Indirect bilirubin	Y	N	
	Direct bilirubin	Y	N	
	G6PD	Y	N	
	BUN	Y	N	
	Creatinine	Y	N	
	Blood group	Y	N	
*** Urine**	Red blood cells	Y	N	
	Haemoglobinuria	Y	N	
	Bilirubinuria	Y	N	
	Urobilirubinuria	Y	N	
	Ketones	Y	N	
	Albuminuria (Protein)	Y	N	
	Nitrites	Y	N	
	Urine sugar	Y	N	
	Urine PH	Y	N	
	Specific gravity (SG)	Y	N	
	Urine leucocytes	Y	N	
	Red blood cell casts	Y	N	